

**The role of sexual selection in adaptation to novel
environments and the effects of environmental change on
sexual selection**

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Statement of originality

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The effect of sexual selection on adaptation and extinction under increasing temperatures.

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Abstract

Sexual selection is ubiquitous in all sexually reproducing species and a powerful evolutionary force. The effect of sexual selection on population fitness has caused wide debate and has been proposed to both enhance adaptation rates, but also possibly increase extinction risk.

Using experimental evolution, the strength of sexual selection was altered by biasing adult sex ratios in replicated populations of the Indian meal moth, *Plodia interpunctella*. Under increasing temperatures strong sexual selection and polyandry was associated with increased population fitness. Suggesting sexual selection could provide a buffer against climate change by increasing adaptation rates. However, no effect on male mating success was observed. In contrast, under stable temperatures male mating success was increased by strong sexual selection, however, this did not translate to increased population fitness. These results indicate that female choice is a potentially strong selective pressure in *P. interpunctella*. Moreover, under stabilising and directional selection the costs and benefits of sexual selection change.

In a field study, dung beetle species richness and abundance were compared across a gradient of habitat disturbance, ranging from old-growth rainforest to oil palm plantation. Species persistence within altered habitats was positively associated with expressing horns and relative horn lengths, suggesting male-male competition increases a species ability to persist within modified landscapes. There was not a strong effect of relative testes mass or sexual size dimorphism on the abundance of species. Additionally, by examining the expression of sexually selected traits within species, horn length and testes mass appear to be condition dependent, but only the expression of horns was effected by habitat change.

Overall, from both laboratory and field studies it was found that sexual selection can increase adaptation rates and the persistence of species within altered and changing environments. Moreover, both sets of studies suggest pre-copulatory sexual selection to be an important aspect of sexual selection in driving this adaptation.

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Chapter 1: General introduction

1.1 Sexual selection

1.1.1 Introduction

Darwin's theory of natural selection (1859) is the fundamental theory of evolutionary biology. The foundation of evolution by natural selection is based upon heritable variation. The variation existing between individuals creates differences in survival and provides a mechanism for natural selection to act, favouring traits which enhance survival and ultimately progeny production within a given environment. Acting over evolutionary timescales natural selection has driven adaptive evolution, often shaping organisms for specific environments (Darwin, 1859). Despite strong directional selection on survival there is a bewildering diversity of traits that are likely to reduce survival and oppose natural selection, such as, bright coloration, extravagant plumage, horns and conspicuous behaviours. Why do such traits evolve and when present in a species why are these traits often only observed in males? In order to answer these questions Darwin proposed a separate theory of sexual selection (Darwin, 1859, 1871).

Sexual selection is defined as “the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction” (Darwin, 1871, pg. 256). This implies a difference in the reproductive success of individuals which is not solely based upon resource acquisition and survival. Generally, males can increase their reproductive success by mating with and successfully inseminating as many females as possible (Bateman, 1948). In contrast, females may increase their reproductive success by mating with one or few ‘high quality’ males (Trivers, 1972). This general distinction between the sexes is caused by anisogamy, the differing sizes of gametes of each sex and their relative

investment in reproduction (Parker, Baker and Smith, 1972). Females invest in relatively few, large costly eggs, whereas males produce many small and motile sperm. The evolution of differences in the behaviour and morphology of the sexes can largely be attributed to this anisogamy (Andersson, 1994).

Generally, the increased investment by females caused by the evolution of anisogamy limits the number of eggs within a population, which the total number of sperm within a population could fertilize many times over, leading to a situation where not all male gametes will fertilise female gametes (Parker, Baker and Smith, 1972; Bulmer and Parker, 2002).

Typically this leads to males competing over fertilisation of a limited number of eggs and drives the differing reproductive strategies of the sexes. This competition can be broadly split into two categories, intrasexual and intersexual selection. Intrasexual selection describes a process whereby individuals of the same sex (usually males) directly compete with each other over access to copulations and fertilization of females, driving the evolution of sexually selected traits such as weaponry or large male body size (Andersson, 1994). Intersexual selection describes a process whereby one sex (usually females) choose members of the opposite sex as mating partners and ultimately which individuals fertilize their gametes, thus driving the evolution of sexually selected traits such as elaborate plumage and courtship display (Andersson, 1994). Furthermore, intrasexual and intersexual selection are not restricted to pre-copulation but can also occur post-copulation in processes termed sperm competition (Parker, 1970) and cryptic female choice (Eberhard, 1996), respectively. Splitting sexually selected traits into specific functional categories is appealing and often useful. However, in reality some sexually selected traits may serve multiple functions, for example in certain systems such as stalk eyed flies, traits used as weaponry during male-male competition may also be favoured by female choice (Burkhardt and de la Motte, 1988; Panhuis and Wilkinson, 1999).

1.1.2 Pre-copulatory sexual selection

Sexual selection may occur before males and females copulate, often causing differences in the quantity and quality of the mating partners of both sexes. A wide diversity of pre-copulatory mechanisms and traits are exhibited in both sexes which act to increase reproductive success. Pre-copulatory sexual selection typically occurs due to males directly competing with each other over access to receptive females or through female choice of attractive males.

1.1.2.1 Intrasexual selection

When a resource is limited, individuals will often compete over access to or monopolisation of it, whether this is food, shelter or mating partners. When considering reproduction, the limiting resource within a population is receptive females because of the differences in investment in reproduction between the sexes (Parker, Baker and Smith, 1972). Competition for access to these mates can potentially skew male reproductive success and exclude certain individuals from gaining copulations. This monopolisation and exclusion could extend over an entire breeding season, and this may be most extreme in lekking species where a few dominant males gain the majority of copulations with females (Emlen & Oring, 1977). As there may be limited opportunity for males to reproduce, competition can lead to aggressive displays and combat, which in some cases has lethal results (Cook, Bean, & Power, 1999).

At the other extreme of male competition it may not be possible for males to exclude or monopolise resources used to attract females or females themselves. All males may then have the potential for equal access to females and males compete indirectly in 'scramble' type competition. In scramble competition it is likely that the need for early detection and swift

locomotion required to find a mate first has driven the evolution of enlarged sensory and locomotory organs in males (Hanks, Millar and Paine, 1996; Bertin and Cézilly, 2003). Scramble competition can also influence life histories, favouring faster development and early maturation in males (Jarošík and Honek, 2007). If many males are present during scrambles, behaviours or traits which hold favourable positions within a swarm or allow privileged access to spawning females are likely to be favoured (McLachlan and Allen, 1987; Petersson, 1987; Brockmann, Nguyen and Potts, 2000).

When resources which attract females or females are economically defensible by males, males may adopt different strategies, territory defence or mate guarding, respectively (Emlen & Oring, 1977). In some cases no physical contact is required during contest. For example, in birds, male contests are often through song which serve to resolve territory disputes (Nowicki *et al.*, 1998; Vehrencamp *et al.*, 2014). Other examples of non-physical territory defence exist in other taxa, for example male butterflies often engage in non-contact aerial contests over territories (Kemp and Wiklund, 2001). When females are unpredictably distributed, males may adopt pre-copulatory mate guarding strategies, guarding immature females until they reach maturity (Elias *et al.*, 2014) or non-breeding females who are only receptive for a short time (Jormalainen, 1998). Mate guarding has been shown not to directly influence mate choice and therefore may represent a male tactic based upon the availability of females (Elias *et al.*, 2014).

During the various types of contest competition increased male body size is often favoured; males with larger bodies tend to have an advantage in physical contests and when ‘sizing up’ (Andersson, 1994). For example, species of non venomous snakes which engage in intrasexual contests tend to show male biased sexual size dimorphism (Shine, 1978, 1994). Exceptions to this appear when there is scramble competition, which tends to favour fast male development and therefore small male body size in order to access females first (Jarošík

and Honek, 2007), or because small size confers advantages in agility and manoeuvrability (McLachlan and Allen, 1987).

In species where contest competition escalates to direct combat, males often possess weaponry (Andersson, 1994). The diversity of weaponry is extremely large and varied in evolutionary origins (Emlen, 2008). Some of the most striking weaponry is found within the ungulates, in the form of horns, antlers and tusks. The use of weaponry is dependent upon its size and shape, typically being used in both offensive and defensive behaviour (Jennings and Gammell, 2013). Male ungulates may engage weaponry during combat in order to defend a harem (Clutton-Brock *et al.*, 1979), territory (Vanpé *et al.*, 2009) or lek (Bro-Jørgensen and Durant, 2003). Many other types of weaponry exist, for example pheasant spurs, *Phasianus colchicus* (Mateos and Carranza, 1996), fiddler crab claws, *Uca pugilator* (Christy and Salmon, 1984), enlarged mandibles in stag beetles (Knell, Pomfret and Tomkins, 2004), the bewildering diversity of horns in dung beetles (Emlen *et al.*, 2005) and many more (reviewed in Emlen, 2008). The function of weaponry appears closely associated with the ability to defend resources which increase the likelihood of gaining mating opportunities and are expected to evolve when reproductive benefits outweigh the costs of bearing the weapon (Emlen, 2008). Furthermore, population density has been argued to be a potentially important factor in whether weaponry will be selected for or against (Knell, 2009b). At low density more investment in traits related to mate searching are likely to be favoured rather than weaponry, whereas at high density those males engaging weapons in aggressive behaviours are expected to pay excessive costs due to increased numbers of rivals. Therefore it is likely that only at intermediate population densities will the reproductive benefits accrued from weaponry outweigh their costs (Knell, 2009b).

If males cannot succeed in direct competition with other males, because for example they have a size disadvantage, there is evidence that alternative mating strategies may be adopted.

Burying beetle, *Nicrophorus vespilloides*, males plastically adopt different mating strategies based upon availability of resources and/or if they have lost in male-male competition (Eggert, 1992). Distinct male colour morphs exist in the fish, *Poecilia parae*, whereby colourful males adopt aggressive behaviours to directly monopolize access to females, whereas the drab coloured morph specialise in a sneak copulation strategy, circumventing both male aggression and female choice (Hurtado-Gonzales and Uy, 2010). Other examples of alternative mating strategies come from dung beetles. Males of many species of *Onthophagus* often develop into two distinct morphs (Emlen, Hunt, & Simmons, 2005), where small minor males have no/small horns and large major males express disproportionally large horns. There is good evidence that majors adopt female monopolisation behaviour in which they guard tunnels dug by females, repeatedly mate with the female and fight with rival males attempting to enter these tunnels, whereas minor males adopt 'sneak' tactics such as digging side tunnels or using agility and intersections in tunnels to bypass competition with the guarding major male and gain access to females (Emlen, 1997a; Moczek & Emlen, 2000).

1.1.2.2 Intersexual selection

Due to the differing investment in gametes (Parker, Baker and Smith, 1972), females with increased investment in reproduction tend to be choosy regarding which male gametes fertilise their large costly eggs (Andersson, 1994). Females can benefit from choice by two mechanisms, direct benefits or indirect benefits. Direct benefits act to increase current female fitness, increasing fecundity or longevity. Indirect benefits act to increase female fitness through their offspring being of higher genetic quality and having increased reproductive success (Andersson and Simmons, 2006).

There are a wide variety of behaviours and resources which can provide direct benefits to females and can include breeding sites and territories, protection from predation, paternal care and nuptial gifts (Møller and Jennions, 2001). In the field cricket, *Gryllus campestris*, male mate guarding has been shown to provide direct benefits to females because when male and female pairs are attacked, males allow female priority to burrows, decreasing the likelihood of females being predated (Rodríguez-Muñoz, Bretman and Tregenza, 2011).

There is widespread evidence that nuptial gifts in insects are associated with increased female fecundity (Vahed, 1998) and females may base their choices upon these nuptial gifts (Wedell and Ritchie, 2004). Interestingly, in a number of tettigoniid species which produce nuptial gifts, sex role reversal occurs when resources are scarce and males impose choice over females due to their investment in a protein rich spermatophore (Gwynne and Simmons, 1990; Simmons and Bailey, 1990).

Females often choose their mates based on sexually selected ornaments and displays (Darwin, 1871; Andersson, 1994). Females may base their mate choices on pre-existing sensory biases, and males may exploit these. For example, in guppies the increased mating success of males with orange colouration may arise because females are attracted to orange coloured objects used during food detection (Rodd *et al.*, 2002). Fisherian mechanisms may also explain female mating preferences, whereby genetic components of female preference and male trait are inherited by both sons and daughters. If a genetic coupling between trait and preference occurs, self-reinforcing coevolution may drive runaway selection (Fisher, 1930). Directional selection as such on traits favoured by choice are however expected to erode variance in male display by driving alleles associated with display to fixation. Females will therefore have little or no choice between males and any benefits of being choosy negated. This has given rise to the so called 'lek paradox' (Kirkpatrick and Ryan, 1991).

A possible resolution the lek paradox was suggested by Rowe & Houle (1996) and has gained wide support as a model of ‘good genes’ sexual selection: the ‘genic capture’ model (Rowe and Houle, 1996). This model has two assumptions: sexually selected traits are condition dependent, and condition has high genetic variance. Condition is expected to be based upon the majority of an individual’s genome, which provides a large target for mutations and deleterious alleles to influence condition. If sexually selected traits are costly and in line with handicap principles of sexual selection (Zahavi, 1975), then only those males in good condition will be able to pay the costs of expressing exaggerated traits. If females base their choice upon these condition dependent sexually selected traits, female offspring will have better genetic quality, providing a mechanism in which females gain indirect benefits of choice, but also variance in male traits under directional selection is maintained (Rowe and Houle, 1996).

There are many examples of females basing their choice on elaborate or exaggerated displays and ornaments in nature and in many cases these traits appear to be honest signals and indicators of male condition and genetic quality. Male courtship rate in dung beetles (Kotiaho, Simmons and Tomkins, 2001; Kotiaho, 2002), ‘song’ in a moth, *Achroia grisella* (Brandt and Greenfield, 2004), eye span in stalked eyed flies, *Cyrtodiopsis dalmanni* (David *et al.*, 2000; Cotton, Fowler and Pomiankowski, 2004a), tail length (Gienapp and Merilä, 2010) and plumage coloration in birds (Keyser and Hill, 1999; Johnsen *et al.*, 2003) and ornamentation in guppies *Poecilia reticulata* (Nicoletto, 1993) have all been shown to be condition dependent.

Not all sexually selected signals, however, may be reliable indicators of genetic quality and condition (Harrison, Thomson, Grant, & Bertram, 2013; Lailvaux, Reaney, & Backwell, 2009). Furthermore, males which do not succeed in seducing females through display or ornamentation may adopt alternative strategies to gain copulations and fertilization success.

For example, males may gain ‘sneaky’ fertilisations and this reproductive strategy has been shown in guppies, *P. reticulata* (Pilastro and Bisazza, 1999; Matthews and Magurran, 2000). Additionally, male *Drosophila* have been shown to force copulations with immature females (Markow, 2000; Seeley and Dukas, 2011) and forced copulations appear to be widespread in waterfowl (Mineau, Derrickson and McKinney, 1983).

1.1.2.3 Polyandry

There are a number of costs associated to females from mating, particularly if females mate with multiple males (Arnqvist and Nilsson, 2000; Lehtonen, Jennions and Kokko, 2012). The costs of mating include increased time, energy and predation risk (Jennions and Petrie, 1997), risk of contracting sexually transmitted disease (Knell and Webberley, 2004) or parasites (Roberts *et al.*, 2015) and physical injury (Crudgington and Siva-Jothy, 2000). Why females are polyandrous and why polyandry is so widespread has caused much debate (Arnqvist and Nilsson, 2000; Simmons, 2003, 2005; Bocedi and Reid, 2015). Polyandrous females may gain both direct and indirect benefits. Some of these benefits include nuptial gifts (Fedorka and Mousseau, 2002), increased likelihood of gametes being genetically compatible (Tregenza and Wedell, 2000) and increased genetic diversity of offspring (Jennions and Petrie, 2000). Furthermore, polyandry causes another selective pressure upon males as the sperm of multiple males compete for fertilisation of eggs. Females are likely to gain further benefits through eliciting sperm competition if male sperm competitiveness is associated with male quality (Simmons and Kotiaho, 2002; Hosken *et al.*, 2008), or sons inherit ‘sexy’ sperm (McNamara, Van Lieshout and Simmons, 2014; Egan *et al.*, 2016)

1.1.3 Post-copulatory sexual selection

Sexual selection does not stop once copulations have been gained but can also occur post-copulation if females mate with multiple males. Polyandry is extremely widespread

throughout animals and therefore post-copulatory sexual selection is likely to be pervasive. Post-copulatory sexual selection is a potentially powerful evolutionary force often occurring unseen within female reproductive tract. Sexual selection can occur when the sperm of multiple males compete to fertilise eggs in sperm competition and females may be able to exert choice over male sperm in cryptic female choice.

1.1.3.2 Sperm competition

In species where females mate with multiple males, the sperm of males compete to fertilise eggs (Parker, 1970). Theory predicts that as the risk (the likelihood that females will remate and sperm will be under competition from a rivals ejaculate) and the intensity (how many ejaculates are competing over fertilisation of eggs) of sperm competition increases selection will favour adaptations which increase male paternity shares (Parker, 1970). Under a ‘fair raffle’ of sperm competition, male fertilisation success will be directly related to the proportion of each males’ sperm which are competing, therefore traits which increase the numbers of male sperm are likely to be favoured (Parker, 1970). There is empirical support from comparative studies that species with increased sperm competition have larger testes (Hosken, 1997; Byrne, Roberts and Simmons, 2002) and evidence from experimental evolution studies that when males evolve with increased risk and intensity of sperm competition selection favours increased testes size (Hosken and Ward, 2001) and larger ejaculates (Ingleby, Lewis and Wedell, 2010). Additionally, in polyphenic dung beetles different male morphs face different levels of sperm competition, such that horned ‘majors’ are able to at least partially monopolise females, whereas hornless ‘minors’, which utilise sneak tactics to gain matings are nearly always mating with previously mated females and therefore under sperm competition (Moczek and Emlen, 2000). This asymmetry in sperm competition between morphs has selected for increased testes investment in minor males

compared to majors (Simmons, Tomkins and Hunt, 1999; Simmons, Emlen and Tomkins, 2007).

Male mating roles are not fixed and males may assume different roles when mating with different females. Males may be mating with virgin females and therefore 'defensive' traits, which protect sperm from a rivals ejaculate will be favoured. Alternatively, males could mate with non-virgin females and therefore 'offensive' traits, which overcome a rival's ejaculate, possibly favoured. Selection is likely to act independently on defensive and offensive traits and theory predicts antagonistic selection (Parker, 1984). Sperm competitiveness is measured through paternity shares, P_1 and P_2 , when in defensive and offensive roles, respectively (Boorman and Parker, 1976). There is empirical evidence of both positive (House and Simmons, 2006) and negative (Bernasconi and Keller, 2001) associations between P_1 and P_2 . Whether males are generally all round good sperm competitors or specialise in specific roles is currently not well understood, but the answer probably relates to male condition and specifics of mating systems and sperm precedence. For example, in insects second male sperm precedence appears to be common and therefore it could be expected that offensive traits will be under stronger selection than defensive traits (Simmons, 2001).

Producing sperm is costly and males mating multiple times may become sperm depleted (Wedell, Gage and Parker, 2002). Males are therefore expected to adjust and tailor their ejaculate to maximise reproductive returns (Drewsbury, 1982). The Indian meal moth, *Plodia interpunctella*, and the small white butterfly, *Pieris rapae*, adjust their ejaculate depending on female mating status and size of rivals' ejaculate, increasing investment in their ejaculate when the risk and intensity of sperm competition is high (Cook & Gage, 1995; Wedell & Cook, 1999). Furthermore, strategic ejaculate allocation patterns have been shown to have evolved in response to increased levels of sperm competition (Ingleby, Lewis and Wedell, 2010).

Sperm competition may also cause selective pressures not only on sperm numbers, but sperm morphology and other components of the ejaculate that may be important in males securing fertilisation success. Sperm size, motility and longevity have been suggested to be important factors in sperm competition (Snook and Karr, 1998; Morrow and Gage, 2000, 2001; Hosken, 2003; Pitnick, Hosken and Birkhead, 2009). Furthermore, other components of the ejaculate exist other than fertile sperm. For example, in Lepidoptera males have two types of sperm, fertile eupyrene sperm and non-fertile apyrene sperm (Friedländer, 1997). The exact function of the apyrene sperm is not well known, but there is evidence of its importance in sperm competition (Gage and Cook, 1994; Watanabe, Bon'no and Hachisuka, 2000; Ingleby, Lewis and Wedell, 2010). In *Pieris napi*, the green-veined white butterfly, apyrene sperm has been shown to increase female refractory period by filling the spermatheca with less costly material than nucleated sperm, thus decreasing risk of sperm competition by increasing the interval between matings (Cook & Wedell, 1999). Ejaculates may also contain other components such as accessory gland proteins (acps), of which some can influence that outcome of sperm competition (Chapman *et al.*, 2000; Chapman, 2001). Males may produce mating plugs (Uhl, Nessler and Schneider, 2009; Sutter *et al.*, 2016), or males may perform post-copulatory mate guarding behaviour (Sakaluk, 1991; Calbacho-Rosa, Córdoba-Aguilar and Peretti, 2010), both of which also act to delay female remating and protect males ejaculate from sperm competition.

1.1.3.2 *Cryptic female choice*

Not only do males' sperm compete for fertilisation when females mate with multiple males, but pre-copulatory female choice is paralleled in the post-copulatory process of cryptic female choice (Eberhard, 1996). In comparison to other aspects of sexual selection the mechanisms of cryptic female choice, in which females bias paternity shares towards specific males, are less well understood. This is likely due to the difficulties of empirical testing

(Birkhead, 1998; Pitnick and Brown, 2000). Despite this, cryptic female is now widely accepted as a potentially powerful evolutionary force with the potential to influence both male and female fitness (Firman *et al.*, 2017).

Sperm storage and usage are a potential mechanism from cryptic female choice. Yellow dung fly, *Scathophaga stercoraria*, females with increased complexity of sperm storage organs have increased control over male ejaculates (Ward, 2001). In the moth, *Ephestia kuehniella*, the spermatophore of the last male to mate displaces the spermatophore of previous males, females therefore could influence how much first male sperm is transferred to the spermatheca by remating sooner (Xu and Wang, 2010). Female fowl, *Gallus gallus domesticus*, have been shown to eject the sperm of subordinate males (Pizzari and Birkhead, 2000) and in the spider, *Physocyclus globosus*, male courtship behaviours influence how much sperm is dumped after copulation (Peretti and Eberhard, 2010). Furthermore, male guppies, *P. reticulata*, which are perceived as attractive by pre-copulatory mechanisms inseminate more sperm and this is independent of any male manipulation (Pilastro *et al.*, 2004).

Evidence exists that sperm-egg interactions could provide another mechanism for cryptic female choice to act. A polymorphism in sperm protein is used in external fertilisation of sea urchin, *Echinometra mathaei* and *Echinometra oblonga*, females to non-randomly select which sperm fertilise eggs based upon both egg and sperm genotypes (Ghaderi *et al.*, 2011). It is also possible that females mediate sperm swimming performance through reproductive fluids. For example, the ovarian fluids of *P. reticulata* can influence sperm velocities and bias the fertilisation success toward unrelated males (Gasparini and Pilastro, 2011).

The difficulties of demonstrating cryptic female choice (Birkhead, 1998; Pitnick and Brown, 2000) mean that the functional significance of cryptic female choice remains difficult to

understand (Firman *et al.*, 2017). However, the evidence that cryptic female choice has the potential to bias the fertilisation success in favour of specific males makes it a potentially powerful mechanism of female choice.

1.2 Sexual conflict

1.2.1 Introduction

Sexual selection acts to increase the reproductive success of individuals and this can cause a conflict between the sexes. Sexual conflict can occur due to one sex increasing their fitness at the expense of the other, or because the sexes share a genome and certain traits have sex specific fitness. These are the two process of sexual conflict, interlocus sexual conflict, conflict arising between alleles at different loci (Chapman *et al.*, 2003), and intralocus sexual conflict, conflict arising between sexes when shared alleles have differing fitness consequences in each sex (Bonduriansky and Chenoweth, 2009; Van Doorn, 2009).

1.2.2 Interlocus sexual conflict

The reproductive interests of males and females rarely coincide and the sexes often have divergent interests in reproduction, as with sexual selection these differences between the sexes are rooted in anisogamy. The examples given when discussing sperm competition; apyrene sperm delaying female remating (Cook & Wedell, 1999) and mating plugs (Uhl, Nessler and Schneider, 2009), are likely to reduce how many males a female will mate with, and if females gain genetic benefits from mating multiple times then this conflict over mating rates may reduce female fitness indirectly. Males may also harass non-receptive females for copulations at the expense of female fitness (Rowe *et al.*, 1994; Ojanguren and Magurran, 2007; Li, Fail and Shelton, 2015). Another common source of sexual conflict comes from parental care. Even in socially monogamous species each sex will be selected to reduce their

costs of raising offspring at the expense of their partners future reproductive success (Trivers, 1972; Houston, Székely and McNamara, 2005). Sexual conflict can also arise in hermaphroditic species where each individual attempts to adopt the less costly male sex role or reduce quantities of sperm transferred (Schärer, Janicke and Ramm, 2014).

Males are sometimes directly harmful to females during mating. For example, *Drosophila* ejaculate contains a cocktail of accessory gland proteins (Acps) which increase male reproductive success but often at the expense to female fitness, increasing female egg laying rate at a cost to longevity and lifetime fecundity (Chapman *et al.*, 1995; Chapman, 2001; Chapman and Davies, 2004; Wigby and Chapman, 2004). Harmful phenotypic traits can be seen in male bed bugs, *Cimex lectularius*, which use specialised intromittent organs to traumatically inseminate females (Stutt and Siva-Jothy, 2001) and the sclerotized spines on the intromittent organ of *Callosobruchus maculatus* (Crudginton and Siva-Jothy, 2000) are both directly harmful to females. Sexual cannibalism exists in spiders and mantids and this is a clear example of sexual conflict, especially in situations where courting males do not gain a copulation before being eaten (Schneider, 2014). The conflict arising from harmful traits can lead to an evolutionary arms race between the sexes, in which one sex increases their ‘manipulation’ while the other sex increases their resistance to ‘manipulation’ (Arnqvist and Rowe, 1995; Rice, 1996, 2000; Wigby and Chapman, 2004).

1.2.3 Intralocus sexual conflict

Due to the sexes sharing a genome, sex specific fitness outcomes of trait expression is constrained by the other sex and can cause neither sex to reach their optima (Bonduriansky and Chenoweth, 2009; Van Doorn, 2009). When the fitness of *D. melanogaster* were assayed for fitness they showed strong sex by genotype interactions, certain genotypes showed relatively high female fitness but when this same genotype was expressed in males there was

a significant reduction in relative fitness and vice versa (Chippindale, Gibson and Rice, 2001). Sexual antagonistic fitness variation has also been demonstrated in red deer, *Cervus elaphus*, whereby males with relatively high fitness sire daughters of relatively low fitness (Foerster *et al.*, 2007). Sexes may have different dietary optima related to sex-specific reproductive needs. However, in the cricket *Teleogryllus commodus* diet choices appear similar between the sexes suggesting some form of antagonistic selection (Maklakov *et al.*, 2008). This genetic tug of war between the sexes could reduce overall population fitness (Arnqvist and Tuda, 2010), however, mechanisms of sex specific gene expression may help to resolve intralocus sexual conflict allowing more optimal sexual dimorphism to be reached (Bonduriansky and Chenoweth, 2009; Van Doorn, 2009).

1.3 Effects of sexual selection and sexual conflict on population fitness

The expression of sexually selected traits are generally associated with costs arising from their expression such that only high quality individuals will be able to survive with such a handicap and therefore sexually selected traits can act as an honest indicator of quality (Zahavi, 1975). The ‘genic’ capture model is a proposed mechanism of sexually selected traits being indicators of genetic quality and posits that the expression of traits are based on the overall condition of the bearer and that condition has high genetic variance (Rowe and Houle, 1996). Male reproductive success is expected to be skewed in favour of individuals with the most exaggerated expression of sexually selected traits that reflect their general health and wellbeing, and thus their underlying genetic quality. This ‘good genes’ model of sexual selection can be applied to all aspects of sexual selection, whereby females base their choice of mates or which males fertilise eggs on condition dependent display traits, or in male-male competition pre- and post-copulation where generally high quality males can exclude individuals in poor condition through competition (Rowe and Houle, 1996).

In many cases, individual deleterious mutations may not influence survival and those that do will be quickly removed from a population via natural selection. Recessive alleles are, however, rarely exposed to selection. Sexual selection through competition or choice acts as a filter on male reproductive success, which if based on the genic capture model, will be proportional to the number of deleterious alleles across all or most of the genome. This has now been recognised as a possible mechanism by which sexual selection could act to increase the purging of deleterious mutations from populations and promote the fixation of beneficial alleles (Whitlock, 2000; Agrawal, 2001; Siller, 2001; Whitlock and Agrawal, 2009). There is now growing empirical evidence that sexual selection increases the removal of deleterious mutations in a population and can increase overall population fitness. Radiation was used to experimentally induced deleterious mutations in populations of dung beetles, *Onthophagus taurus*, and in those populations in which sexual selection was allowed, the purging of these mutations was increased and likely reflects the condition dependence of sexually selected traits skewing reproductive success towards individuals with fewer deleterious mutations (Almbro and Simmons, 2014). Other evidence comes from a long term experiment in red flour beetles, *Tribolium castaneum* (Lumley *et al.*, 2015). By experimentally enforcing inbreeding which exposes deleterious recessive alleles to selection, Lumley *et al.* (2015) showed that those populations which had been evolving with strong sexual selection had increased reproductive fitness and overall lineage survival compared with populations evolving with weak sexual selection. These results suggest that the years of increased strength of sexual selection was increasing the rate of removal of deleterious mutations from populations.

In contrast to sexual selection increasing the average fitness of a population (Jarzebowska and Radwan, 2010; Almbro and Simmons, 2014; Lumley *et al.*, 2015), there are also examples which show that sexual selection has no effect on the removal of mutation load

(Radwan *et al.*, 2004; Arbuthnott and Rundle, 2012). The failure of sexual selection to increase purging of the genome of deleterious mutations in these cases has been argued to be caused by sexual conflict (Arbuthnott and Rundle, 2012). The effect of harmful interactions between the sexes and sexually antagonistic selection could be detrimental to overall population productivity and experimental removal of sexual conflict by enforcing monogamy on populations has been shown to increase net reproductive rate compared to polygamous populations (Holland and Rice, 1999; Fricke and Arnqvist, 2007): this could potentially counteract any increase in population mean fitness from increased sexual selection in polyandrous populations.

The cost of expressing sexually selected traits and the other associated factors such as reducing effective population sizes (Kokko and Brooks, 2003) have also been shown to be detrimental to overall population fitness. Much of this evidence comes from field studies (Doherty *et al.*, 2003; Morrow and Pitcher, 2003; Bro-Jørgensen, 2014) and suggests that when animals are not within controlled laboratory conditions the costs of sexual selection can increase the extinction risk of populations. Horn length in bovids and sexually dichromatic plumage and testes size in birds have all been shown to be positively associated with extinction risk (Doherty *et al.*, 2003; Morrow and Pitcher, 2003; Bro-Jørgensen, 2014).

The possible positive and negative effects of sexual selection to overall population fitness have led researchers to question the effect sexual selection may have on populations when facing novel environment factors (Candolin and Heuschele, 2008; Holman and Kokko, 2013). The increased costs and load imposed by sexually selected traits within novel environments have been theoretically shown to increase extinction risk (Tanaka, 1996), and this increased load may be particularly detrimental to the survival of small population (Martínez-Ruiz and Knell, 2016). Evidence that sexually dichromatic bird species have decreased survival compared to monochromatic species after introduction to islands corroborate these findings

(McLain, Moulton and Redfearn, 1995; Sorci, Møller and Clobert, 1998; McLain, Moulton and Sanderson, 1999). The costs of sexual conflict have also been shown to retard a population's adaptive capacity to novel environments (Holland, 2002; Rundle, Chenoweth and Blows, 2006; Chenoweth *et al.*, 2015), probably due to harmful male traits reducing population sizes and the genetic diversity of populations important in adaptation (Chevin, Lande and Mace, 2010; Hoffmann, Sgrò and Kristensen, 2017).

Counter to all these arguments that sexual selection and the associated effects of sexual conflict decrease population fitness in novel environments, the condition dependence of sexually selected traits could increase adaptation rates. If trait expression is based upon the overall condition of the bearer, then sexual selection should skew reproductive success in favour of animals which currently have the most adaptive alleles within an altered environment, thus promoting the spread of adaptive alleles (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016). There are a number of empirical examples corroborating these arguments of sexual selection increasing adaptation rates (Fricke and Arnqvist, 2007; Long, Agrawal and Rowe, 2012; Plesnar-Bielak *et al.*, 2012; Jacomb, Marsh and Holman, 2016). A number of these examples also come from species in which sexual conflict and male harm are known to reduce population fitness under stabilising selection (Fricke and Arnqvist, 2007; Long, Agrawal and Rowe, 2012), suggesting that when sexual selection is based upon adaptive and maladaptive alleles, the benefits of sexual selection can outweigh the costs of sexual conflict (Long, Agrawal and Rowe, 2012).

One area of research that has received no empirical investigation, but has been suggested to potentially be important (Candolin and Heuschele, 2008) and theoretically shown to have an effect (Martínez-Ruiz and Knell, 2016), is whether the degree and speed of environmental change influences the relationship between sexual selection and adaptation rates, population persistence and extinction risk. Furthermore, which aspects of sexual selection are driving

these effects has rarely been investigated. It is these questions that are addressed within this thesis.

1.4 Outline and aims of thesis

The first aim of this thesis is to address the effects of sexual selection on species adaptation and population persistence within novel and changing environments. I approach this in two ways: the first using experimental evolution techniques in a laboratory setting; and the second using a field based comparative study. The second aim within these respective systems was to identify which aspects of sexual selection could be driving any effects of sexual selection on population fitness and how habitat change may influence the expression of sexually selected traits.

In chapter 2 I investigate evolved and plastic responses to sexual selection under both stabilising and directional selection. Replicated experimental evolution populations of the Indian meal moth, *Plodia interpunctella*, with male biased and female biased sex ratios and therefore differing strengths of sexual selection were established. These experimental evolution populations were then either exposed to stable or increasing temperatures to simulate possible effects of climate change. Furthermore, when assessing fitness, the phenotypic effects of sexual selection and sexual conflict were manipulated by assigning individuals to either polyandrous or enforced monogamous mating systems, respectively.

Chapter 3 investigates which mechanisms of sexual selection may be influencing population fitness under stabilising and directional natural selection by using the same experimental evolution populations in chapter 2 to assess the strength of pre- and post-copulatory sexual selection in males. Males from both increasing temperatures and stable temperatures were assayed for pre-copulatory reproductive quality by performing mating assays and post-copulatory success estimated from testes dissections.

A field study was carried out in chapter 4 to investigate whether sexual selection can drive adaptation or increase extinction risk within altered habitats. This was done by comparing the persistence and abundance of dung beetles with varying intensities of sexual selection, across a habitat disturbance gradient ranging from tropical old-growth forest to oil palm plantation. The strength of sexual selection was estimated in each species by phenotypic traits often used as surrogate indices sexual selection; these include the presence and absence of horns, relative horn length, sexual size dimorphism and relative testes mass.

In chapter 5 I selected a number of dung beetles species which are likely to have ecological importance, based on body sizes and overall abundance, to investigate how habitat modification influences the expression of sexually selected traits. These results are discussed in the context of condition dependent trait expression and overall population fitness.

Chapter 2: The effect of sexual selection on adaptation and population fitness under increasing and stable temperatures in *Plodia interpunctella*

2.1 Abstract

Strong sexual selection has been reported to both enhance and hinder the adaptive capacity and persistence of populations when exposed to novel environments. Consequently, how sexual selection influences population adaptation and persistence under stress remains widely debated. Here are presented two empirical investigations of the fitness consequences of sexual selection on populations of the Indian meal moth, *Plodia interpunctella*, exposed to stable or gradually increasing temperatures. When faced with increasing temperatures strong sexual selection was associated with both increased fecundity and offspring survival compared to populations experiencing weak sexual selection, suggesting sexual selection acts to drive adaptive evolution by favouring beneficial alleles. Strong sexual selection did not, however, delay extinction when the temperature became excessively high. By manipulating individuals' mating opportunities during fitness assays the effect of multiple mating independently from the effect of population-level sexual selection was able to be assessed, and it was found that polyandry has a positive effect on both fecundity and offspring survival under increasing temperatures. Within stable temperatures there were some benefits from sexual selection but these were not consistent across the entire experiment, possibly reflecting changing costs and benefits of sexual selection under stabilising and directional selection. These results indicate that sexual selection can provide a buffer against climate change and increase adaptation rates although within a continuously changing environment the positive effects of sexual selection may be too small to protect populations and delay extinction when environmental changes are relatively rapid.

2.2 Introduction

Some models of global climate change predict increases of over 4°C in mean global temperature within the next century with associated increases in extreme weather events (Collins *et al.*, 2013). The impact of climate change on ecosystems and biodiversity remains difficult to predict but it is likely that many species across the globe will be affected. Some populations could shift geographical ranges to track favourable environments (Williams *et al.*, 2008) but this is unlikely to be an option for many, especially island species and those within fragmented habitats. These trapped populations will need to adapt *in situ* to their changing environments or potentially face population decline and extinction. Adaptation to environmental change may occur via phenotypic plasticity or genetic change (Hoffmann and Sgro, 2011) but the degree of plasticity available in most populations is likely to be limited and in most cases some degree of genetic change via natural selection will be necessary to allow persistence. Despite recent attempts to understand adaptation and extinction risk during environmental change (Chevin, Lande and Mace, 2010), it has been highlighted that our current understanding of the underlying mechanisms which influence the adaptive capacity and vulnerability of species is poor (Williams *et al.*, 2008; Huey *et al.*, 2012). One such poorly understood mechanism with broad implications is the effect of sexual selection on adaptation rates and extinction risk (reviewed in Candolin & Heuschele, 2008; Holman & Kokko, 2013).

Sexual selection is ubiquitous in sexually reproducing organisms, caused by competition between individuals over access to copulations and fertilizations. It is a potent evolutionary force responsible for many of the more dramatic traits found in nature (Darwin, 1871; Andersson, 1994). An obvious effect of sexually selected traits is to enhance the competitiveness and/or attractiveness of the bearer (usually and hereafter males) and thereby

increase their pre- and post-copulatory reproductive success (Andersson, 1994). Sexually selected traits are often, however, costly for males to produce and maintain, frequently moving individuals away from their naturally selected optima (Darwin, 1871; Andersson, 1994) and for example, can increase predation risk (Godin and McDonough, 2003) and metabolic costs (Allen and Levinton, 2007). The costs and benefits associated with sexual selection are not restricted to males and can also influence the fitness of the sex which does not possess sexually selected traits (usually and hereafter females). Females can benefit from mate choice or multiple mating via both direct and indirect benefits. These can act to increase female fecundity or longevity, or offspring viability and sexual quality, respectively (Andersson, 1994). There are also costs associated with female choice and mating with multiple males, such as, increased male harassment (Rowe *et al.*, 1994; Li, Fail and Shelton, 2015) and exposure to harmful male traits (Chapman *et al.*, 1995).

The varying costs and benefits of sexual selection associated with individual fitness have caused wide debate regarding the overall effect of sexual selection on population viability (Tanaka, 1996; Whitlock, 2000; Agrawal, 2001; Siller, 2001; Kokko and Brooks, 2003; Lorch *et al.*, 2003; Candolin and Heuschele, 2008; Martínez-Ruiz and Knell, 2016) and its potential implications for applied conservation and biodiversity management (Sorci, Møller and Clobert, 1998; Doherty *et al.*, 2003; Morrow and Fricke, 2004; Bro-Jørgensen, 2014). Unsurprisingly, emphases on different aspects of sexual selection have led to predictions of both positive and negative effects of sexual selection on population fitness and viability, highlighting the complex nature of sexual selection. One potentially important effect of sexual selection on populations is caused by ‘good genes’ mechanisms of sexual selection which can, theoretically, increase adaptation rates (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016).

There is now growing consensus and evidence that in many cases mating success appears to be based upon the condition-dependence of sexually selected traits which reflect the underlying genetic quality of the bearer via the ‘genic capture’ mechanism (Rowe and Houle, 1996; Kotiaho, Simmons and Tomkins, 2001; Tomkins *et al.*, 2004; Cotton, Small and Pomiankowski, 2006). In these systems sexual selection can influence mean population fitness by skewing reproductive success in favour of males with more beneficial alleles and/or fewer deleterious mutations than the overall population average (Whitlock, 2000; Agrawal, 2001; Siller, 2001). These effects are likely to be especially important during environmental change: Lorch *et al.* (2003) proposed that those individuals most suited to the new environment will have the greatest expression of condition-dependent sexually selected traits, skewing reproductive success in their favour. As a consequence of the increased reproductive success experienced by these ‘high quality’ males, sexual selection can increase the proportion of alternative beneficial alleles in a population and enhance adaptation rates to novel and changing environments (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016). Recent empirical work has provided support for these ideas, with populations of beetles exposed to a novel diet (Fricke and Arnqvist, 2007) or to a pesticide (Jacomb, Marsh and Holman, 2016) showing reduced adaptation when sexual selection was experimentally reduced. Similarly, monogamous populations of bulb mites, *Rhizoglyphus robini*, had decreased fitness and a greater tendency to go extinct compared to polygamous populations when exposed to a raised temperature (Plesnar-Bielak *et al.*, 2012). Strong sexual selection has also been shown to lead to faster removal of experimentally induced deleterious mutations (Almbro and Simmons, 2014; but also see Arbuthnott and Rundle, 2012; Power and Holman, 2015) and to reduce extinction rates when populations are inbred, as seen in bulb mites (Jarzebowska and Radwan, 2010) and flour beetles (*Tribolium castaneum*) (Lumley *et al.*, 2015).

Moving to the field, a series of studies (McLain, Moulton and Redfearn, 1995; McLain and Vives, 1998; Sorci, Møller and Clobert, 1998; Doherty *et al.*, 2003; Bro-Jørgensen, 2014) indicate that rather than increasing population level fitness, the associated costs of sexual selection may instead increase the extinction risk of a population. For example, comparative studies of birds have shown that species with sexually dichromatic plumage, an indicator of sexual selection, are less likely to successfully colonise islands after introduction (McLain, Moulton and Redfearn, 1995; Sorci, Møller and Clobert, 1998) and have increased localised extinction rates (Doherty *et al.*, 2003) compared with species with monochromatic plumage. Additionally, horn length, a sexually selected trait indicating the strength of sexual selection experienced in bovid species (Bro-Jørgensen, 2007), is positively correlated with IUCN extinction risk status (Bro-Jørgensen, 2014), although across mammals sexual size dimorphism and testes size show no pattern with extinction probability (Morrow and Fricke, 2004). This discrepancy between laboratory and field data might be explained by a recent model by Martínez-Ruiz and Knell (2016) which showed that the inherent costs of sexually selected traits can make small populations particularly prone to extinction during environmental change due to demographic stochasticity but further work is needed to test this hypothesis.

To add further complexity to the overall effect of sexual selection on population viability it is possible that sexual conflict (Chapman *et al.*, 2003; Bonduriansky and Chenoweth, 2009; Van Doorn, 2009) could influence population mean fitness and adaptive capacity. When sexual conflict was removed from populations by enforcing monogamy, populations have been shown to have increased net reproductive rate compared to polygamous populations in which sexual conflict was still present (Holland and Rice, 1999; Fricke and Arnqvist, 2007). Increased adaptation rates arising from sexual selection and genic capture could therefore be countered by sexual conflict, which can potentially retard the adaptive capacity of

populations because traits selected for male reproductive success act antagonistically to female fitness (i.e. fecundity) (Holland, 2002; Rundle, Chenoweth and Blows, 2006; Chenoweth *et al.*, 2015). Rundle *et al.* (2006) reported that this conflict load hindered adaptation to a novel diet in the fruit fly, *Drosophila serrata*, and behavioural observations provided evidence that high quality females suffer disproportionately from female-condition male-induced-harm, reducing the contribution of adaptive beneficial alleles from females to future generations (Chenoweth *et al.*, 2015). Similarly, sexual selection was ineffective in influencing evolutionary adaptation to thermal stress in *D. melanogaster*, suggesting either that female choice was non-adaptive or that any benefits of sexual selection were negated by sexual conflict (Holland, 2002).

Predicting the effect of sexual selection on adaptation and population viability during environmental change therefore remains difficult and the mixed results from previous studies make it hard to discern general trends. All previous experimental work has used step changes in an environmental factor to explore the influence of sexual selection on adaptation rates (Holland, 2002; Rundle, Chenoweth and Blows, 2006; Fricke and Arnqvist, 2007; Plesnar-Bielak *et al.*, 2012; Reding, Swaddle and Murphy, 2013; Chenoweth *et al.*, 2015; Jacomb, Marsh and Holman, 2016). Testing the role of sexual selection on adaptation within a gradually changing environment, which in many cases is likely to represent a more realistic model of environmental change on populations as directional environmental change often occurs across multiple generations (i.e. air temperature or ocean pH: Collins *et al.*, 2013; Rhein *et al.*, 2013) and therefore an important area for experimental investigation.

Furthermore, empirical work using *Drosophila* has shown sexual conflict to be a potentially important factor in determining the outcome of sexual selection on adaptation (Holland, 2002; Rundle, Chenoweth and Blows, 2006; Chenoweth *et al.*, 2015). Although sexual conflict is likely to be pervasive in sexually reproducing species, the widespread use of

Drosophila in studies of sexual selection and adaptation is possibly skewing current perspectives.

Here are presented a set of experiments using the model species the Indian meal moth, *Plodia interpunctella*, a pest of stored food products with wide global distribution. *P. interpunctella* is a relatively short lived polygamous species with a ‘scramble competition’ mating system. Both sexes have relatively low mating rates (Cook, 1999; Ingleby, Lewis, & Wedell, 2010). Mating begins with females advertising their position and receptivity to males through pheromones (Brady *et al.*, 1971). Attracted males then perform a courtship display consisting of rapidly fanning their wings and producing ultrasonic pulses (Trematerra and Pavan, 1995). Importantly, mating will not occur unless a female accepts a male by raising her abdomen which allows copulation to begin (personal observation). There is no evidence that males have traits which directly harm females during copulation, however, occasionally males will harass unreceptive females (personal observation), if and how harmful this is to females is unknown. Additionally, the ejaculate of *P. interpunctella* contain both fertile and non-fertile sperm, the latter may be involved in conflict over mating rates and act to decrease female remating (Cook & Wedell, 1999; Ingleby *et al.*, 2010), reduced female remating is likely to cause indirect costs to females by decreasing the genetic diversity of offspring. Furthermore, there is evidence of intra-locus sexual conflict in a number of shared life history traits (Lewis, Wedell, & Hunt, 2011). *P. interpunctella* represents a model species in which female choice and sperm competition are both likely to be important components of sexual selection. There is the potential for inter-locus sexual conflict to be occurring and evidence of intra-locus sexual conflict (Lewis *et al.*, 2011), however no evidence exists that *P. interpunctella* males directly harm females during mating.

These experiments set out to test the effects of strong and weak sexual selection on population fitness under both stabilising and directional natural selection, with populations

exposed to stable temperatures in one experiment and to gradually increasing temperatures in the other experiment. It is predicted that strong sexual selection will skew reproductive success in favour of high quality males and we will observe an increase in offspring survival and female fecundity in male biased populations compared to female biased populations.

Whether populations reside close to their adaptive peaks or not will possibly alter the relative costs and benefits of sexual selection. If the costs and benefits of sexual selection are altered by environmental change we predict to see differences between temperature experiments in the effect of evolving with strong or weak sexual selection. Predicting the direction of this effect however is difficult and will be determined by the net effect of costs and benefits of sexual selection. If there is a net positive benefit of strong sexual selection to populations, under increasing temperatures we predict that male biased populations will have a delayed extinction and survive increasing temperatures longer than the female biased populations.

To gain further insight into the phenotypic effects of sexual selection on individual female fitness, during fitness assays in both experiments the mating system was manipulated to remove or allow sexual selection, by enforcing monogamy or allowing mate choice, polyandry and consequently any potential inter-locus sexual conflict, respectively. If inter-sexual conflict is intense we predict to see reduced fecundity and longevity of polyandrous females compared to females enforced to monogamy, alternatively, if males are relatively benign and do not harm females during mating we predict there to be no effect of polyandry on fecundity and female longevity. Independent of this, polyandry is predicted to increase offspring survival as females have the opportunity to choose the highest quality male and gain indirect genetic benefits.

2.3 Methods

2.3.1 Animal husbandry

A large outbred stock population of *P. interpunctella* was cultured from multiple laboratory cultures to increase genetic variation. Larvae were reared on an excess of standard diet of bran midlings, yeast and glycerol at a ratio of 10:1:1, and all life stages were maintained at 27°C with a 12:12 light:dark cycle. The stock population was maintained at an approximate even sex ratio with non-overlapping generations.

2.3.2 Experimental evolution populations

Sixteen replicate experimental evolution populations were established from the outbred stock population six generations after it was established following similar protocols outlined in Ingleby et al (2010). Experimental evolution populations were reared on an excess of the standard diet *ad libitum* to remove any potential food competition between larvae and with the same photoperiod as the outbred stock population.

There were two treatments which altered the strength of sexual selection by manipulating the adult sex ratio; strong sexual selection with a male biased adult sex ratio (MB; 3 males: 1 female) and weak sexual selection with a female biased adult sex ratio (FB; 1 male: 3 females), each treatment replicated eight times. 120 adults at the stated adult sex ratio were used to found each population and subsequently establish the next generations. Similar designs with sexual selection manipulated by altering sex ratios have been used in previous studies and Lumley et al (2015) demonstrated that the heterozygosity of populations with similar effective population sizes used in this experiment were unaffected.

Adult sex ratios and populations were maintained by separating 5th instar larvae according to sex to ensure virginity and housing larvae in same sex pots, after eclosion all virgin adults

were placed into egg collecting pots and after 72hrs eggs were collected which established the next generation (Figure 2.1). To maintain similar larval conditions approximately equal numbers of eggs were used from both MB and FB treatments. Once possible to sex larvae (the testes of male 5th instar larvae are clearly visible on the dorsal side through the body wall), collections were performed three times over a week in order to gain moths to both maintain populations and to be used in fitness assays (see below). At stable and slightly increased temperatures a surplus of larvae were collected, however, at increased temperatures the number of larvae decreased. Larvae were allocated at a ratio of 3:1 to maintain populations and experimental fitness assays, respectively. Our experimental design ensured a random representation of eggs, larvae and adults, which is proportional to the reproductive success of both males and females from the previous generation, in both the maintenance of populations and fitness assays.

Each population was also assigned to either the stable temperature experiment (27°C) or to the increasing temperature experiment, in which the temperature was increased by 2°C every other generation starting from 27°C (i.e. $F_{1+2} = 27^{\circ}\text{C}$, $F_{2+3} = 29^{\circ}\text{C}$, etc.), creating four replicate populations for each sexual selection treatment within each experiment.

Temperatures were maintained using ten incubators made from expanded polystyrene boxes and heat mats, each containing a small fan and controlled by a thermostat, all incubators were kept within a control temperature room at 27°C. Using data loggers the temperature of each incubator was recorded every 10 minutes to ensure correct temperatures were achieved and maintained $\pm 0.5^{\circ}\text{C}$. To remove any incubator effect and pseudoreplication every week incubators were randomly reassigned to stable or increasing temperature experiments. Additionally, experimental evolution populations and experimental moths were moved between incubators of the same temperature experiment daily.

2.3.3 Fitness assays

In both experiments a set of fitness assays were performed every generation from F₃ onwards and were carried out at the temperature currently experienced by the population within their respective experiment. Larvae were separated by sex and housed in same sex pots to ensure virginity. Only virgin adults < 24 hours old were used during fitness assays and were housed in 30ml vials for the duration of their lives. During fitness assays an extra treatment was included to investigate phenotypic effects of sexual selection on individual female fitness. In both the stable and increasing temperature experiments moths from both strong and weak sexual selection treatments (MB and FB, respectively) were randomly assigned to either monogamous (1 male: 1 female) or polyandrous (3 males: 1 female) mating system treatments (Figure 2.1).

Female lifetime fecundity was determined every 48hrs by moving all surviving moths to a new vial and counting eggs from the old vial under magnification. Offspring fitness was determined by estimating proportion offspring survival, measured as eggs surviving to adulthood. A subset of 20 eggs were taken from each female and placed on an excess of food, if less than 20 eggs were laid all eggs were used. The number of emerged adults were counted and sexed after 6 weeks. The development time (egg to adult) of *P. interpunctella* is approximately 4 weeks (Lewis et al., 2011), pots were also checked for any pupa not yet eclosed (which were never found) to ensure all surviving offspring were counted. The longevity of all the moths used during fitness assays were recorded and all the offspring which survived to adulthood were sexed.

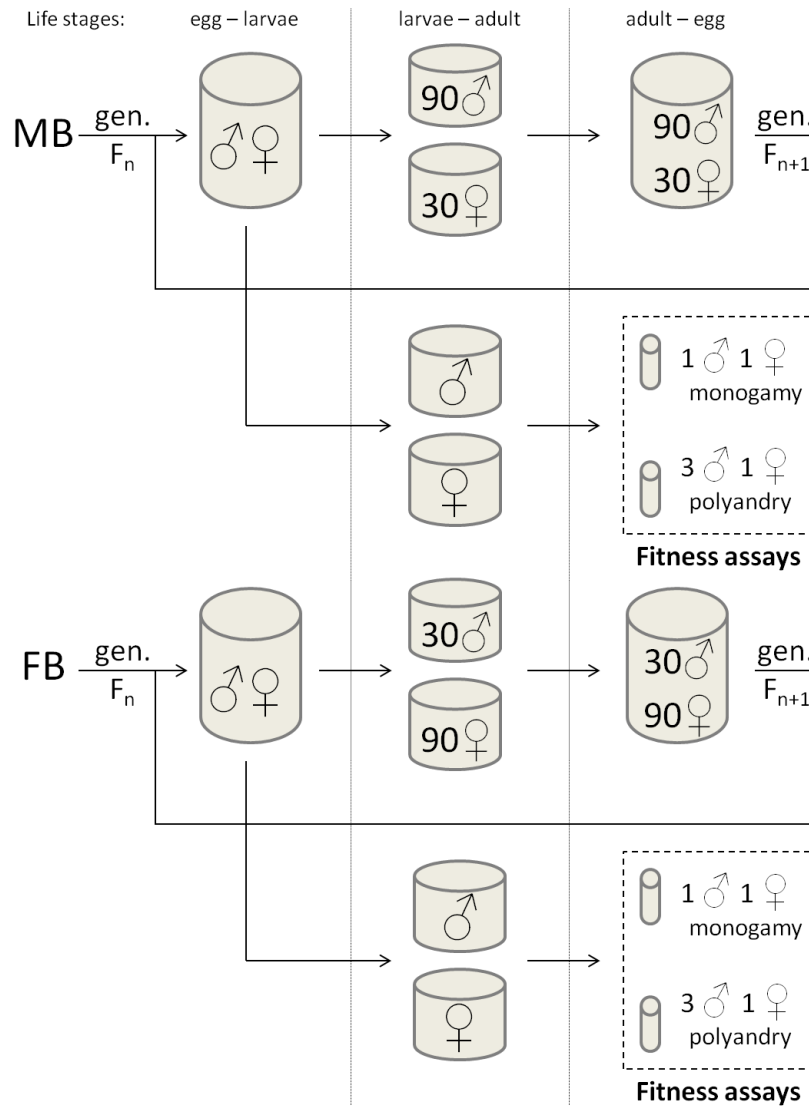


Figure 2.1: Schematic of experimental design showing the maintenance of both strong (male biased; MB) and weak (female biased; FB) sexual selection treatments. The dashed boxes show that during fitness assays moths from both MB and FB populations were placed in monogamous and polyandrous mating systems, which removed and allowed sexual selection to act on individuals, respectively. Experimental design ensured a random representation of eggs, larvae and adults (which is proportional to the reproductive success of both males and females from the previous generation) in both maintenance of populations and those used during fitness assays.

2.3.4 Statistical analysis

All statistical analysis was carried out using R statistical software version 3.3.0 (R Development Core Team, 2016). Model simplification was performed by removing non-significant terms until a minimal adequate model was reached and residuals checked for heteroscedasticity. All analysis was performed using general and generalized mixed effects models with the R package *lme4* (Bates *et al.*, 2015) with population as a random effect to account for variance from repeated measures within and across generations. The analyses were identical between temperature experiments, unless stated otherwise. Direct comparison between the different temperature experiments should be made with caution as statistical analysis was carried out within and not directly between temperature treatments.

Population persistence to increasing temperatures was analysed using the total number of adults available to found the next generation. Moths used to maintain population gives an indication of the general productivity of the entire population. A generalised linear mixed effect model with Poisson error structure was fitted to population size data with sexual selection treatment (MB or FB) and generation as a factor, including their interaction term as explanatory variables.

Generalised linear mixed effects models with Poisson and binomial error structures were fitted to the fecundity and offspring survival data, respectively. To account for overdispersion in these models an observation level random effect was also included. Standard linear mixed effects models were fitted to longevity data, which was log transformed to reduce heteroscedasticity. To provide insight into female reproductive strategy the number of eggs laid after 96 hours as a proportion of total fecundity was also calculated for each female and generalised linear mixed effects models with binomial error structure were fitted to sex ratio of surviving offspring and proportion of total eggs laid after 96 hours data. Due to extremely

low offspring survival in generation F_8 in the increasing temperature experiment, fitting models to the full increasing experiment data set caused model convergence issues, therefore analysis was performed on F_{3-7} only for offspring survival and sex ratio data.

Generation was fitted as a factor rather than a continuous explanatory variable because exploratory analysis demonstrated that it was difficult to fit curves which gave a good fit to data from the increasing temperature experiment without using rather complex nonlinear models. Because generation was fitted as a factor temperature was not used as an explanatory variable in the increasing temperature experiment. In all models sexual selection treatment (MB or FB), mating system treatment (monogamy or polyandry) and generation, plus interaction terms up to the third order were used as explanatory variables. To account for pseudoreplication of male longevity from polyandrous mating system treatments an extra random effect of vial number was included in analyses of male longevity.

2.4 Results

2.4.1 Increasing temperature experiment

2.4.1.1 Population survival

All populations experiencing an increasing temperature became extinct when the temperature reached 35°C at generation 9. There was no effect of the strength of sexual selection on population persistence in the face of increasing temperatures, indicated by a non-significant interaction between sexual selection treatment and generation ($\chi^2 = 12.27$, $df = 1$, $p = 0.140$; Figure 2.2 and Table 2.1). The strength of sexual selection did, however, have significant effects on both fecundity and offspring survival before population extinction.

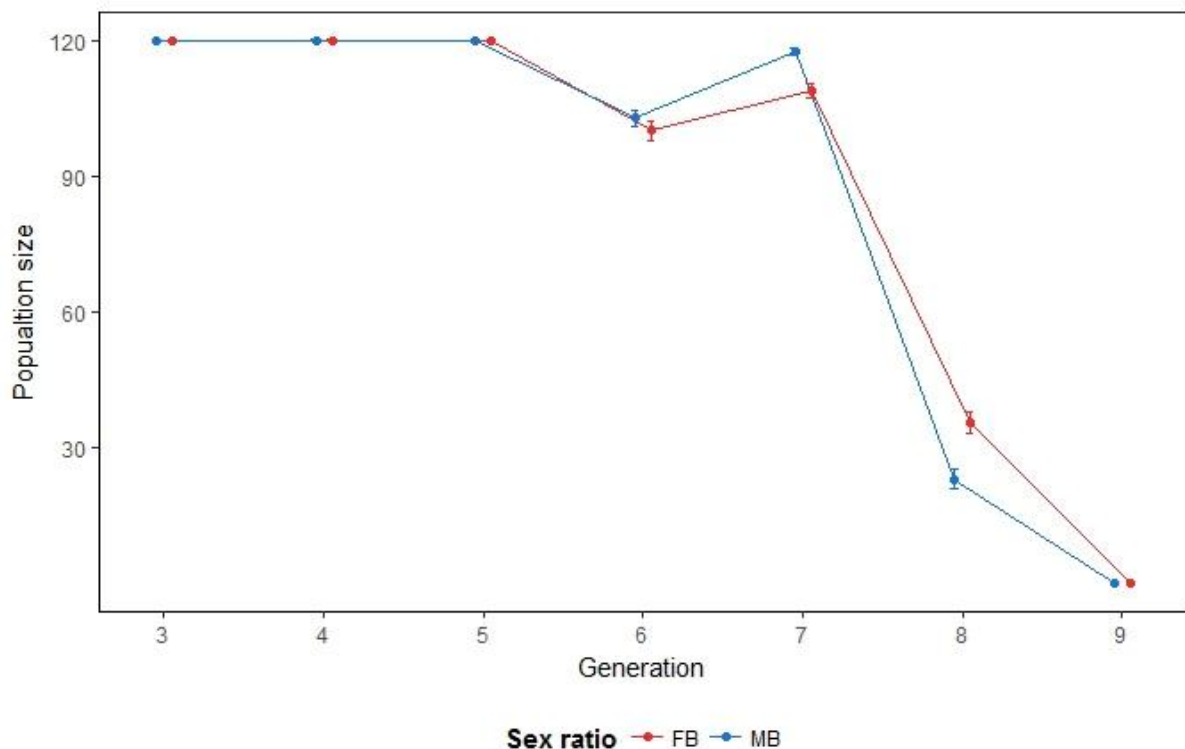


Figure 2.2: Survival of female biased (FB: red) and male biased (MB: blue) populations from the increasing temperature experiment across F_{3-9} . Points are mean (\pm SE) total number of adults used at each generation to maintain populations.

Table 2.1: Results from fitting a generalised linear mixed effects model with Poisson error structure to predict the survival of populations from the increasing temperature experiment.

The asterisk * indicates an interaction term between factors.

Source of variation	Population survival		
	χ^2	df	<i>p</i>
Sexual selection	0.00	1	0.989
Generation	2291.6	1	< 0.001
Sexual selection * Generation (as factor)	12.27	1	0.140

2.4.1.2 Offspring survival

The minimal adequate model fitted to the offspring survival data retained the two-way interaction between sexual selection treatment (MB or FB) and mating system treatment (monogamy or polyandry) ($\chi^2 = 6.2$, $df = 1$, $p = 0.013$; Figure 2.3; Table 2.2). Additionally, the main effect of generation was also retained ($\chi^2 = 220.25$, $df = 1$, $p < 0.001$). The latter arises from a clear effect of increasing temperature (as indicated by generation) in reducing offspring survival, with offspring survival rates also depending on both the sexual selection and mating system treatments. When monogamous mating was enforced during the fitness assays, MB populations showed consistently higher offspring survival than FB populations, especially in the first generation after a temperature increase (figure 2.3). Under enforced monogamy MB offspring survival was on average 8% greater than FB offspring survival across all generations. When polyandrous mating was allowed in the fitness assays, however, this increase in offspring survival found with strong sexual selection was largely reduced, and MB and FB populations showed near-identical reductions in offspring survival as the temperature increased.

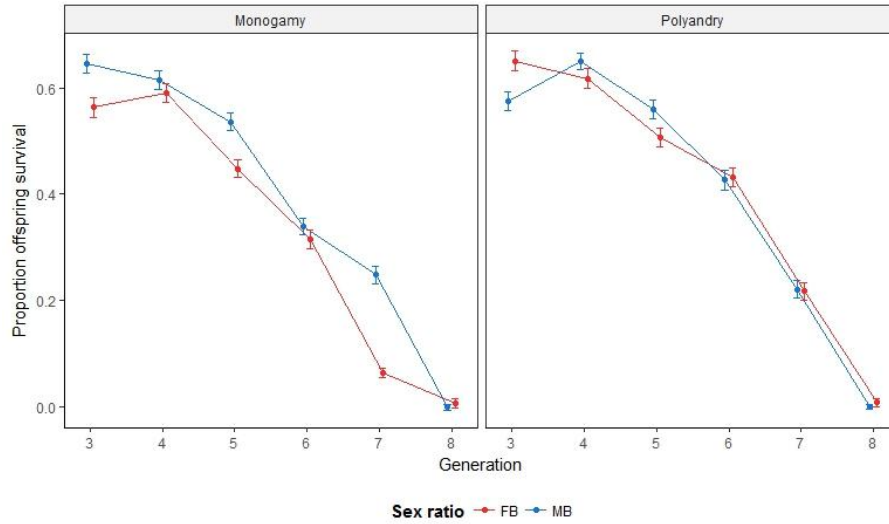


Figure 2.3: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on proportion offspring survival from the increasing temperature experiment across F_{3-8} . Error bars indicate standard error.

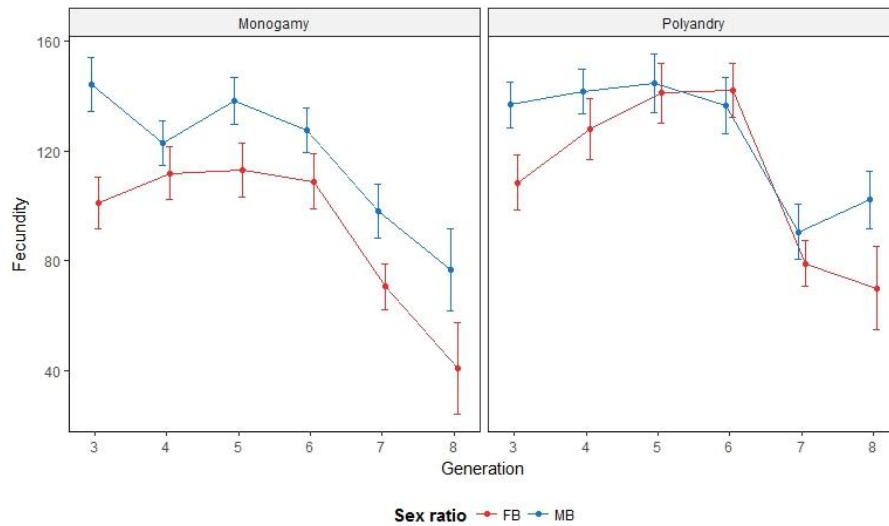


Figure 2.4: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on lifetime female fecundity from the increasing temperature experiment across F_{3-8} . Error bars indicate standard error.

2.4.1.3 Fecundity

The minimal adequate model fitted to the fecundity data showed similar effects, retaining both the two-way interactions between sexual selection and mating system treatments ($\chi^2 = 3.88$, $df = 1$, $p = 0.049$; Figure 2.4; Table 2.2) and mating system and generation ($\chi^2 = 13.08$, $df = 1$, $p = 0.023$). Under enforced monogamy MB females consistently had a higher fecundity compared to FB females, laying 32 more eggs each on average. Allowing polyandry during fitness assays tended to increase the fecundity of FB females and the observed difference in MB and FB fecundity when under enforced monogamy largely disappeared. Inspection of figure 2.4 and model summary indicates that the interaction between mating system treatment and generation is largely driven by polyandrous females having increased fecundity in generation F₈ only, and therefore no overall meaningful pattern can be discerned. Upon further inspection however, fecundity was only reduced once temperatures were increased by 6°C in generations F₇ and F₈.

2.4.1.4 Female longevity

Female longevity decreased throughout the experiment, indicated by a main effect of generation ($\chi^2 = 250.49$, $df = 1$, $p < 0.001$; Figure 2.5; Table 2.2). Furthermore, female longevity was notably reduced by strong sexual selection, with females from MB populations living for approximately 1 day fewer than those from FB populations ($\chi^2 = 64.27$, $df = 1$, $p < 0.001$). Mating system had a similar effect on female longevity and females exposed to a polyandrous mating system also lived for approximately 1 day fewer than monogamous females ($\chi^2 = 90.55$, $df = 1$, $p < 0.001$).

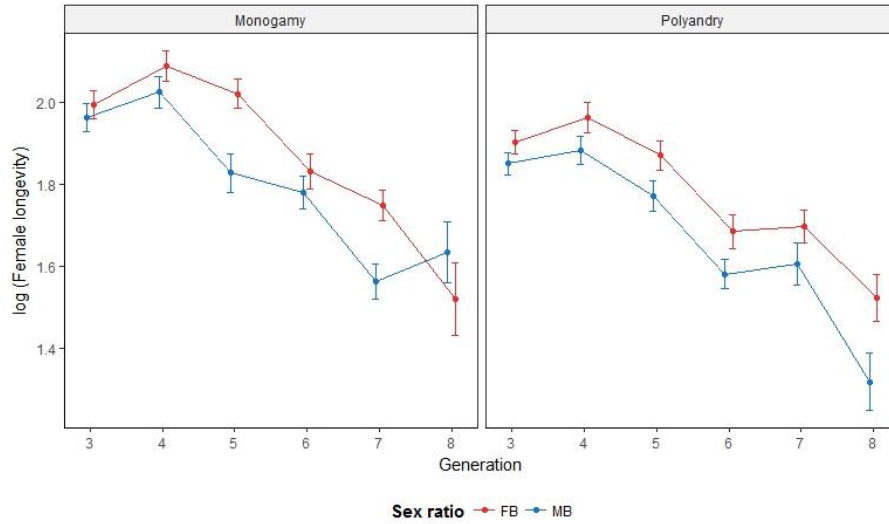


Figure 2.5: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on log transformed female longevity from the increasing temperature experiment across F_{3-8} . Error bars indicate standard error.

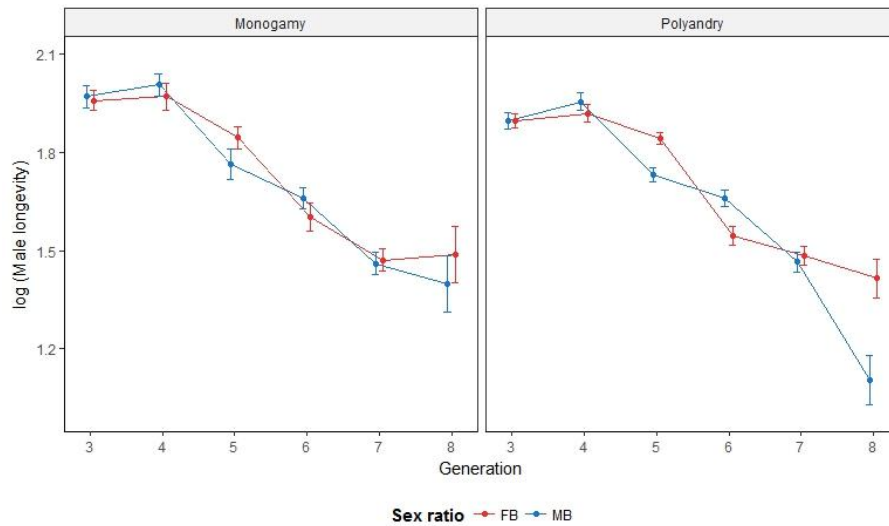


Figure 2.6: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on log transformed male longevity from the increasing temperature experiment across F_{3-8} . Error bars indicate standard error.

2.4.1.5 Male longevity

Male longevity also tended to decrease throughout the experiment. Overall, the polyandrous mating system treatment significantly reduced male longevity compared to males under enforced monogamy ($\chi^2 = 5.65$, $df = 1$, $p = 0.018$; Figure 2.6; Table 2.2). A significant two-way interaction between generation and sexual selection treatment (MB or FB) was also retained in the minimal adequate model ($\chi^2 = 26.86$, $df = 1$, $p < 0.001$), suggesting that the sexual selection treatment influences male longevity. This interaction seems largely to arise from differences within individual generations however, making general patterns hard to discern from the fitted model.

2.4.1.6 Sex ratio of offspring

There was a general lack of variation in the sex ratio of offspring surviving to adulthood and none of the explanatory variables in the initial model were significant (Figure 2.7; Table 2.2). There was a close to significant effect of mating system treatment, suggesting that offspring of females from polyandrous mating systems tended towards being females biased.

2.4.1.7 Egg laying rate

The two-way interaction between mating system and generation was retained in the minimal adequate model fitted to proportion of total eggs laid after 96 hours data ($\chi^2 = 11.35$, $df = 1$, $p = 0.023$; Figure 2.8; Table 2.2). Furthermore, there was a significant main effect of sexual selection ($\chi^2 = 19.84$, $df = 1$, $p < 0.001$). Overall a reasonably clear pattern is shown in figure 2.8 in most generations females placed in a polyandrous mating have an increased egg laying rate compared to monogamous females, with the significant two-way interaction being largely driven by further increased egg laying rate of polyandrous females in generation F₆. Additionally, MB females have an increased egg laying rate compared to FB females,

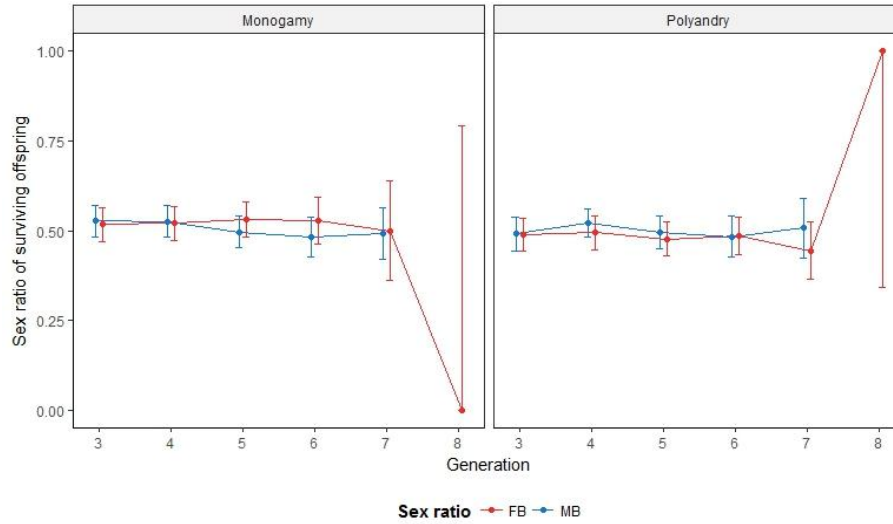


Figure 2.7: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on sex ratio of surviving offspring (0 = all female; 1 = all male) from the increasing temperature experiment across F_{3-8} . Error bars indicate standard error.

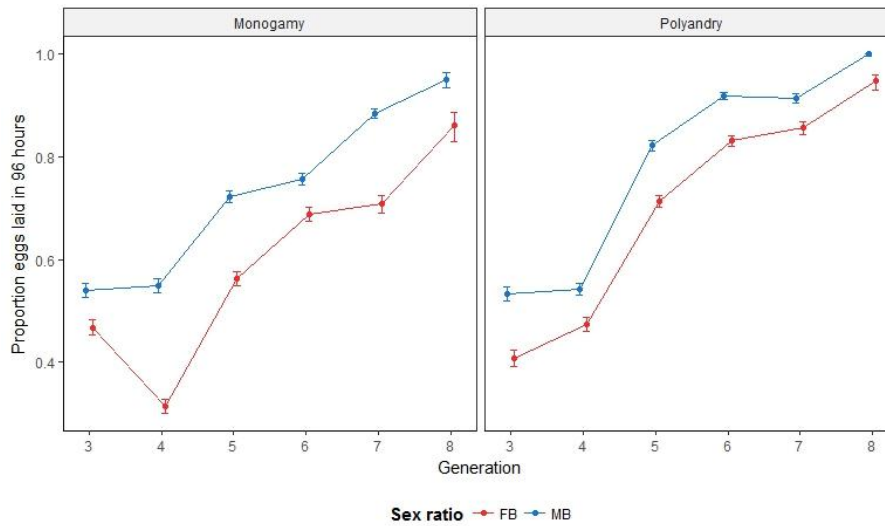


Figure 2.8: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on proportion of total eggs laid in 96 hours from the increasing temperature experiment across F_{3-8} . Error bars indicate standard error.

Table 2.2: Results from fitting mixed effects model to predict proportion offspring survival, fecundity, log transformed female longevity, log transformed male longevity, sex ratio of offspring surviving to adulthood and the proportion of total eggs laid after 96 hours from the increasing temperature experiment. Analysis of proportion offspring survival, sex ratio of offspring surviving to adulthood and the proportion of total eggs laid after 96 hours was performed on data from F₃₋₇ only. The asterisk * indicates an interaction term between factors.

Source of variation	Proportion offspring survival			Fecundity		
	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
Generation (as factor)	220.25	1	<0.001	-	-	-
Mating system	-	-	-	-	-	-
Sexual selection	-	-	-	-	-	-
Mating system * Generation (as factor)	4.21	1	0.378	13.08	1	0.023
Sexual selection * Generation (as factor)	3.25	1	0.517	8.55	1	0.129
Sexual selection * Mating system	6.20	1	0.013	3.88	1	0.049
Sexual selection * Mating system * Generation (as factor)	5.25	1	0.263	2.78	1	0.734

Source of variation	log (female longevity)			log (male longevity)		
	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
Generation (as factor)	250.49	1	<0.001	-	-	-
Mating system	39.40	1	<0.001	5.65	1	0.018
Sexual selection	13.13	1	<0.001	-	-	-
Mating system * Generation (as factor)	10.34	1	0.066	6.34	1	0.275
Sexual selection * Generation (as factor)	6.38	1	0.271	25.74	1	<0.001
Sexual selection * Mating system	0.00	1	0.980	0.11	1	0.741
Sexual selection * Mating system * Generation (as factor)	8.47	1	0.132	3.06	1	0.691

Source of variation	Sex ratio of offspring surviving to adulthood			Proportion eggs laid in 96 hours		
	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
Generation (as factor)	2.74	1	0.603	-	-	-
Mating system	3.72	1	0.054	-	-	-
Sexual selection	0.06	1	0.806	19.84	1	<0.001
Mating system * Generation (as factor)	0.33	1	0.988	11.35	1	0.023
Sexual selection * Generation (as factor)	1.81	1	0.771	2.79	1	0.593
Sexual selection * Mating system	1.81	1	0.179	0.19	1	0.661
Sexual selection * Mating system * Generation (as factor)	1.14	1	0.888	7.92	1	0.095

2.4.2 Stable temperature experiment

2.4.2.1 Offspring survival

When temperatures were stable throughout the experiment, analysis of the offspring survival data retained a significant two-way interaction between sexual selection treatment (MB or FB) and generation ($\chi^2 = 26.47$, $df = 1$, $p < 0.001$; Figure 2.9; Table 2.3). This two-way interaction arises because although there were differences in the survival of MB and FB offspring between generations, these differences were not consistent across the entire experiment. In contrast, having the opportunity for mate choice and multiple mating within the polyandrous mating treatment tends to increase offspring survival ($\chi^2 = 5.91$, $df = 1$, $p = 0.015$). Across the entire experiment polyandrous females had, on average, a 7% higher offspring survival compared to females forced to mate monogamously.

2.4.2.2 Fecundity

Fecundity was influenced by the mating system treatment, but only in a few generations ($F_{5,7} \& 8$), indicated by a two-way interaction between mating system and generation ($\chi^2 = 14.75$, $df = 1$, $p = 0.012$; Figure 2.10; Table 2.3). There was no effect of sexual selection treatment on female fecundity ($\chi^2 = 0.01$, $df = 1$, $p = 0.952$).

2.4.2.3 Female longevity

Female longevity tended to decrease throughout the experiment ($\chi^2 = 161.58$, $df = 1$, $p < 0.001$; Figure 2.11; Table 2.3). There was a significant main effect of mating system treatment, females from polyandrous mating systems tended to live for approximately 1 day fewer than monogamous females ($\chi^2 = 69.56$, $df = 1$, $p < 0.001$). There was no effect of sexual selection treatment on female longevity ($\chi^2 = 0.36$, $df = 1$, $p = 0.546$).

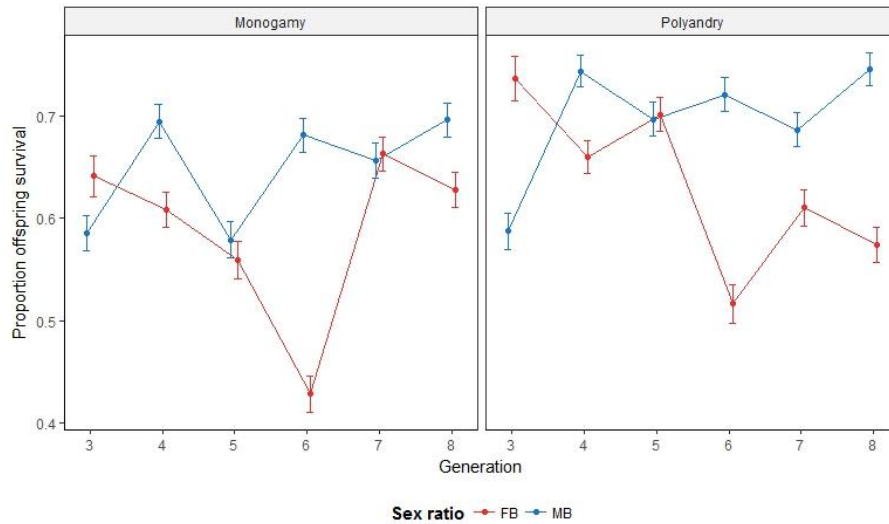


Figure 2.9: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on proportion offspring survival from the stable temperature experiment across F_{3-8} . Error bars indicate standard error.

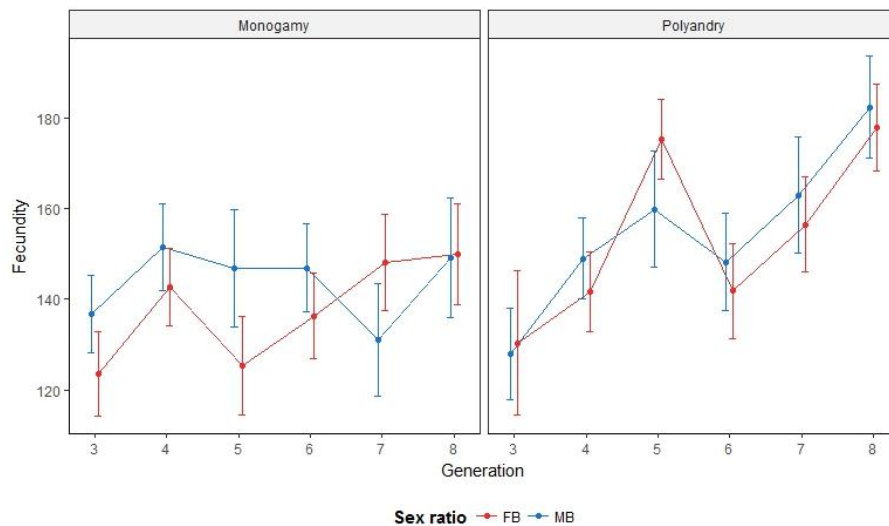


Figure 2.10: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on lifetime female fecundity from the stable temperature experiment across F_{3-8} . Error bars indicate standard error.

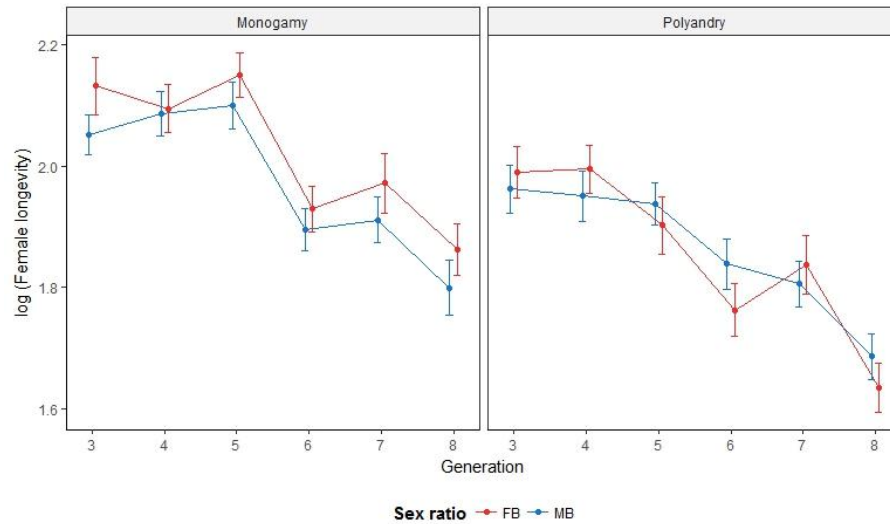


Figure 2.11: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on log transformed female longevity from the stable temperature experiment across F_{3-8} . Error bars indicate standard error.

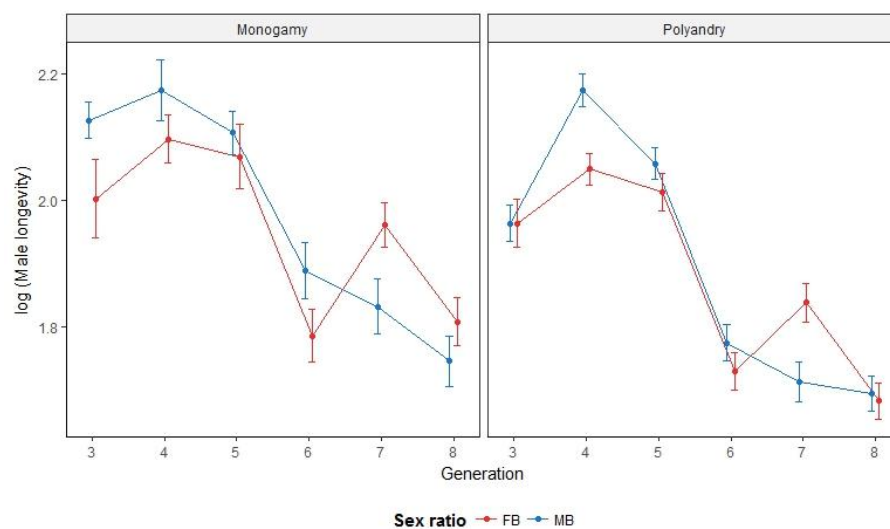


Figure 2.12: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on log transformed male longevity from the stable temperature experiment across F_{3-8} . Error bars indicate standard error.

2.4.2.4 Male longevity

There was also a general trend for male longevity to decrease throughout the experiment. The significant two-way interaction between generation and sexual selection treatment was retained in the minimal adequate model ($\chi^2 = 21.43$, $df = 1$, $p < 0.001$; Figure 2.12; Table 2.3) and largely driven by differences within generations, meaning general patterns are difficult to discern. Male longevity was also reduced by the polyandrous mating system in comparison to the longevity of males under enforced monogamy ($\chi^2 = 21.68$, $df = 1$, $p < 0.001$).

2.4.2.5 Sex ratio of offspring

The sex ratio of offspring surviving to adulthood on average did not deviate from approximately an equal sex ratio. None of the explanatory variables were significant (Figure 2.13, Table 2.3).

2.4.2.6 Egg laying rate

The proportion of total eggs laid by females in 96 hours was found increase in the latter half of the experiment as indicated by a main effect of generation ($\chi^2 = 112.07$, $df = 1$, $p < 0.001$; Figure 2.14; Table 2.3). A significant main effect of mating system treatment was also retained in the minimal adequate model ($\chi^2 = 33.66$, $df = 1$, $p < 0.001$), showing that polyandrous females have an increased egg laying rate compared to monogamous females. There was no effect of sexual selection treatment ($\chi^2 = 0.07$, $df = 1$, $p = 0.792$).

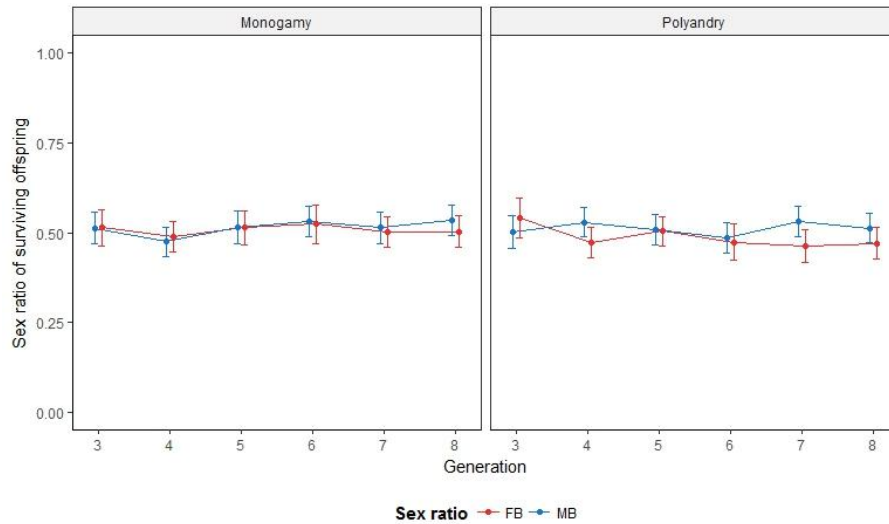


Figure 2.13: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on sex ratio of surviving offspring (0 = all female; 1 = all male) from the stable temperature experiment across F_{3-8} . Error bars indicate standard error.

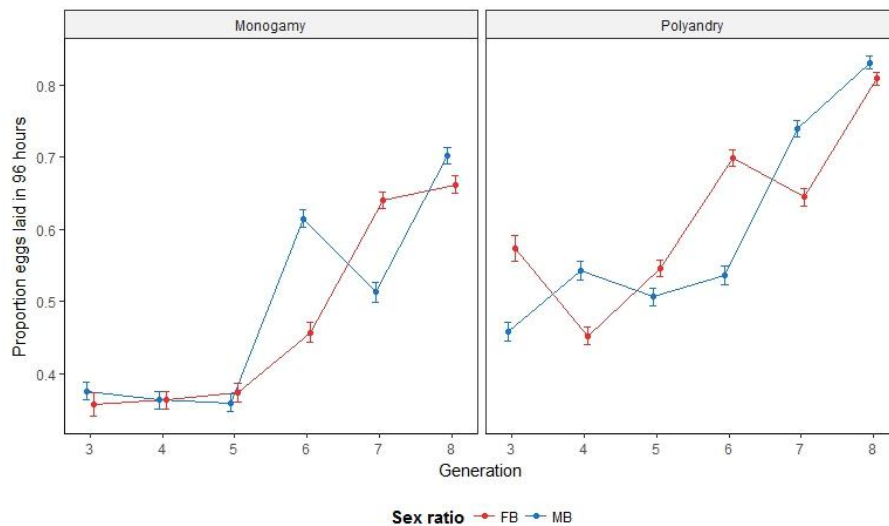


Figure 2.14: Comparisons of the effect of weak and strong sexual selection (FB; red or MB; blue, respectively) and mating system (monogamy; left panel or polyandry; right panel) treatments on proportion of total eggs laid in 96 hours from the stable temperature experiment across F_{3-8} . Error bars indicate standard error.

Table 2.3: Results from fitting mixed effects model to predict proportion offspring survival, fecundity, log transformed female longevity, log transformed male longevity, sex ratio of offspring surviving to adulthood and the proportion of total eggs laid after 96 hours from the stable temperature experiment from F₃₋₈. The asterisk * indicates an interaction term between factors.

Source of variation	Proportion offspring survival			Fecundity		
	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
Generation (as factor)	-	-	-	-	-	-
Mating system	5.91	1	0.015	-	-	-
Sexual selection	-	-	-	0.01	1	0.952
Mating system * Generation (as factor)	8.83	1	0.116	14.75	1	0.012
Sexual selection * Generation (as factor)	26.47	1	<0.001	2.45	1	0.784
Sexual selection * Mating system	0.01	1	0.936	0.19	1	0.666
Sexual selection * Mating system * Generation (as factor)	4.76	1	0.446	5.71	1	0.336

Source of variation	log (female longevity)			log (male longevity)		
	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
Generation (as factor)	161.58	1	<0.001	-	-	-
Mating system	69.56	1	<0.001	21.68	1	<0.001
Sexual selection	0.36	1	0.546	-	-	-
Mating system * Generation (as factor)	4.92	1	0.426	3.82	1	0.576
Sexual selection * Generation (as factor)	2.22	1	0.818	21.43	1	<0.001
Sexual selection * Mating system	3.09	1	0.079	0.06	1	0.800
Sexual selection * Mating system * Generation (as factor)	2.73	1	0.742	4.22	1	0.518

Source of variation	Sex ratio of offspring surviving to adulthood			Proportion eggs laid in 96 hours		
	χ^2	df	<i>p</i>	χ^2	df	<i>p</i>
Generation (as factor)	1.96	1	0.855	112.25	1	<0.001
Mating system	1.48	1	0.224	33.64	1	<0.001
Sexual selection	1.53	1	0.216	0.07	1	0.792
Mating system * Generation (as factor)	6.00	1	0.306	5.35	1	0.375
Sexual selection * Generation (as factor)	3.74	1	0.588	2.32	1	0.804
Sexual selection * Mating system	0.95	1	0.330	0.12	1	0.728
Sexual selection * Mating system * Generation (as factor)	4.05	1	0.542	6.91	1	0.227

2.5 Discussion

Within the increasing temperature experiment we observed a clear effect of increasing temperatures decreasing the viability and the overall production of populations, mainly arising from decreased offspring survival. This decrease in offspring survival was, however, reduced in the strong sexual selection treatment compared to the weak sexual selection treatment, but only when monogamy was enforced during mating assays. Furthermore, we also found that those females from populations experiencing strong sexual selection had an increased fecundity compared to females from the weak sexual selection treatment. Overall these results suggest that sexual selection is acting to enhance the promotion of beneficial alleles within a population and facilitate adaptation to increasing temperatures through ‘good genes’ mechanisms of sexual selection. These results are in agreement with previous empirical studies and our predictions, suggesting that if condition-dependence of sexually selected traits is based upon the genic capture model (Tomkins *et al.*, 2004), sexual selection can improve population fitness (Hollis, Fierst and Houle, 2009; Jarzebowska and Radwan, 2010; Almbro and Simmons, 2014; Lumley *et al.*, 2015) and enhance adaptation (Fricke and Arnqvist, 2007; Plesnar-Bielak *et al.*, 2012; Jacomb, Marsh and Holman, 2016). In this case, however, and against our prediction the improved fitness associated with strong sexual selection was not sufficient to delay extinction as temperatures became higher.

The pre- and post-copulatory reproductive success of male *P. interpunctella* is likely to be based on many behaviours and broad physiology, which the genic capture model predicts will be affected by the overall health and wellbeing of an individual and based upon many loci across much of the genome. We did not directly assess the strength of condition dependence of sexually selected traits, but we did find that the longevity of males placed in a polyandrous mating system during fitness assays was consistently reduced across both experiments.

Sexual selection is known to be costly to males and has been shown experimentally to reduce male longevity in a number of taxa (e.g. Clutton-Brock & Langley, 1997; Kotiaho & Simmons, 2003; Partridge & Farquhar, 1981); these costs are likely to increase with the presence of rivals (Callander, Kahn, Hunt, Backwell, & Jennions, 2013; Cook & Gage, 1995) and possibly explain the observed reductions in male longevity. Costly traits are expected to be condition-dependent (Rowe and Houle, 1996) and the genic capture model offers a probable mechanism to explain the observed increase in offspring survival and female fecundity in those populations exposed to increasing temperatures.

In comparison and as predicted we observed a difference in the effect of strong sexual selection under stable and increasing temperatures. We found much less evidence of an evolutionary effect of sexual selection on offspring survival and female fecundity within the stable temperature experiment compared to the increasing temperature experiment. Previous work has suggested that when populations are close to their adaptive peaks the benefits of sexual selection may be outweighed by the cost imposed through sexual conflict (Fricke and Arnqvist, 2007; Long, Agrawal and Rowe, 2012). The difference in the effect of strong sexual selection on fecundity and offspring survival between stable and changing environments might be caused by similar mechanisms since intra-locus sexual conflict within *P. interpunctella* is known to occur (Lewis et al., 2011), but see Willis (2015). Alternatively, the beneficial effects of sexual selection may be small and hard to detect when populations reside close to their adaptive peaks. Across both experiments however, the sex ratio of offspring surviving to adulthood did not deviate from approximately 50:50. Assuming an equal sex ratio of eggs laid this suggests that within both benign and stressful environments intra-locus sexual conflict does not have a strong affect on offspring survival and its effect on overall population viability in this system appears minimal. Similarly, there was no evidence of inter-locus sexual conflict negatively affecting either population or individual female

fitness in the way that has been described from some other systems (Holland and Rice, 1999; Holland, 2002; Rundle, Chenoweth and Blows, 2006; Fricke and Arnqvist, 2007; Long, Agrawal and Rowe, 2012; Chenoweth *et al.*, 2015). In fact females placed within a polyandrous mating system gained both direct and indirect benefits which increase fecundity and offspring viability, respectively. These beneficial effects of polyandry were more consistent within the increasing temperature experiment, possibly reflecting changing costs and benefits of sexual selection to individuals residing close to and away from their adaptive peaks (Long, Agrawal and Rowe, 2012).

The increased fecundity observed in polyandrous females appears to be related to changes in reproductive allocation (Sheldon, 2000). Across both experiments polyandrous females showed an increased egg laying rate over the first 96hrs of their lives which appears to be at the expense of longevity. *P. interpunctella* adults do not feed and it appears that they are following a ‘live fast, die young’ strategy: when allowed to choose a mate they appear to be investing more resources in reproduction than when monogamy is enforced, leading to higher reproductive output concentrated in a shorter life. Polyandry may also provide insemination advantages by increasing the genetic compatibility of gametes (Tregenza and Wedell, 2000). Furthermore, in a variety of taxa males provide nuptial gifts to females which increase resources for female egg construction (Vahed, 1998), and it is possible polyandrous females have derived some form of nutrient from the ejaculate of multiple males. We predicted that if males were relatively benign to females while mating and gaining copulations we would see no effect of polyandry on fecundity, the fact we observed overall increases in fecundity due to polyandry suggests that any costs due to inter-locus sexual conflict are minimal in *P. interpunctella* and clearly surpassed by the positive effects polyandry has to female fitness.

In both experiments we predicted and observed polyandry increasing offspring survival, a process which has been shown a number of times (Simmons, 1987; Fedorka and Mousseau,

2002; García-González and Simmons, 2011), and which is likely a consequence of both female choice and sperm competition in *P. interpunctella* being based upon male condition. There is evidence that pre- and post-copulatory reproductive success are not associated in this system (Lewis, Lizé, & Wedell, 2013) and the relative importance of pre- and post-copulatory processes in determining the genetic benefits within the strong sexual selection and polyandrous mating system treatments is unknown. Despite this, it is clear that genetic benefits of polyandry exist and are important to individual female fitness in *P. interpunctella*; this is most obvious when comparing the offspring survival of polyandrous and monogamous females from FB populations when stressed and exposed to increasing temperatures.

As population sizes are reduced by increasing temperatures there is likely a corresponding reduction in population genetic diversity and increased inbreeding, potentially causing populations to enter an extinction vortex (Gilpin and Soulé, 1986). In contrast to Plesnar-Bielak et al (2012) the positive evolutionary effects of strong sexual selection in the present study were unable to delay this process and the persistent increases in temperature drove all populations to extinction. This indicates that the rate of temperature increase was greater than the critical rate of environmental change (Chevin, Lande and Mace, 2010) and that even with strong sexual selection adaptation was not swift enough to allow survival. Worryingly the rate of increase of temperature in this experiment is comparable to those which longer-lived species with slow generation times are predicted to experience under climate change (Collins *et al.*, 2013).

We predicted that if strong sexual selection increased offspring survival under increasing temperatures then due to this enhanced adaptation we would observe MB populations surviving at higher temperatures than FB populations, contrary to this prediction no difference between the survival of MB and FB populations was observed. If the temperature increases were halted at approximately 31°C or if the rate of temperature increase was

reduced strong sexual selection might have delayed the extinction of MB populations, for two reasons. Firstly, we observed evolutionary effects of strong sexual selection increasing both female fecundity and offspring viability at increasing temperatures, suggesting that adaptation was indeed occurring faster within MB populations; and secondly, there are clear direct and indirect benefits of polyandry within the increasing temperature experiment. Given that females from MB populations are generally polyandrous, whereas females from FB populations are likely to only mate once (Ingleby, Lewis and Wedell, 2010), the observed benefits of polyandry would on average be more pronounced in populations under strong sexual selection. Understanding how variation in sexual fitness is effected by differing rates and degrees of environmental change remain important areas for future studies to address (Candolin and Heuschele, 2008).

Overall, using experimental evolution we have shown that strong sexual selection can improve offspring fitness in relatively few generations when populations are exposed to stable temperatures. Furthermore, we observed clear positive evolutionary effects of strong sexual selection when populations are exposed to increasing temperatures. These benefits did not, however, delay the extinction of populations exposed to increasing temperatures and possibly due to the relatively large and often changes in temperature. Additionally, we find a strong effect of polyandry on individual female fitness, especially within increasing temperatures. This strongly suggests that benefits of female choice and multiple mating may provide a buffer against and increase a population's resilience to changing environments and climate change. We suggest that the degree of polyandry and strength of sexual selection should be a consideration in applied conservation and for those working to negate the impacts of climate change on biodiversity, especially when assessing potential impacts on a range of species with differing mating systems.

Chapter 3: The effects of sexual selection on pre- and post-copulatory sexually selected traits in *Plodia interpunctella* under increasing and stable temperatures

Dr Alice Laughton, Jahcub Trew and Anthony Johnson provided assistance with mating assays.

3.1 Abstract

Sexual selection is expected to favour males of high genetic quality and therefore possibly increase the expression of sexually selected traits within populations. Whether environmental change alters the expression of sexually selected traits may provide insight into condition dependence and which aspects of sexual selection are under strong selection. Populations of the Indian meal moth, *Plodia interpunctella*, were maintained under conditions of strong and weak sexual selection, and exposed to stable and increasing temperatures. In order to assess how strength of sexual selection and environment affected evolution in these populations, I estimated pre-copulatory sexual selection through mating assays, and post-copulatory sexual selection by measuring the relative size of testes of males from each population. Under stable temperatures males from populations with strong sexual selection have an increased mating success compared to males from populations with weak sexual selection; however no overall pattern in relative testes size was found. This suggests that males are facing stronger selection from female choice than sperm competition in *P. interpunctella* when populations are under stabilising selection. Mating assays performed with males reared under increasing temperatures no longer detected a difference in the mating success of males from populations experiencing strong and weak sexual selection. This suggests environmental change alters the expression of pre-copulatory sexually selected traits. Similarly no strong overall patterns in

relative testes size were found, although there is some evidence that at high temperatures males experiencing weak sexual selection have relatively larger testes than those males with experiencing strong sexual selection. This is counter to what was expected and possibly caused by large variation between generations in relative testes size. These results are discussed in the context of previous fitness assays estimating the effect of sexual selection on population fitness and adaptation rates.

3.2 Introduction

Anthropogenic driven environmental change is a major concern for biodiversity. The widespread changes in environmental factors occurring today (Collins *et al.*, 2013) are likely to cause a mismatch between organisms' phenotypes and the environment in which they exist. This mismatch may cause declines in populations and possible extinction (Burger and Lynch, 1995). In order to avoid extinction populations will need to adapt and therefore understanding mechanisms which may influence adaptation and extinction are important areas for research.

Sexual selection is the process in which individuals compete over copulations and fertilisations, ultimately determining an individual's fitness. Sexual selection is a potent evolutionary force and responsible for driving the evolution of some of the most extravagant and conspicuous morphologies and behaviours found in nature (Andersson, 1994). The effects of sexual selection are diverse and can act congruently and antagonistically with individual non-sexual fitness, which can potentially influence population fitness under both stabilising and directional natural selection (Candolin and Heuschele, 2008).

Theoretical work has suggested that sexual selection can enhance the fixation of beneficial alleles (Whitlock, 2000), reduce mutation load (Agrawal, 2001; Siller, 2001) and increase adaptation rates (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016). A number of recent studies have confirmed this empirically and shown that sexual selection increases population fitness within both stable (Hollis, Fierst and Houle, 2009; Jarzebowska and Radwan, 2010; Lumley *et al.*, 2015) and novel environments (Fricke and Arnqvist, 2007; Plesnar-Bielak *et al.*, 2012; Jacomb, Marsh and Holman, 2016). Many of the arguments used to explain these findings are based upon 'good genes' sexual selection and genic capture (Tomkins *et al.*, 2004). The genic capture model posits that the expression sexually selected traits are based

upon the condition of the bearer and that condition is based upon the overall health and vigour of the individual (Rowe and Houle, 1996). The expression of sexually selected traits should therefore reflect the underlying genetic quality of the individual and their suitability to current environmental conditions. By skewing reproductive success in favour of 'high quality' individuals sexual selection may act to increase population fitness (Whitlock, 2000; Lorch *et al.*, 2003).

Sexually selected traits are generally costly to produce and maintain and there is evidence that males suffer increased mortality rates due to the expression of sexually selected traits (Promislow, 1992; Promislow, Montgomerie and Martin, 1992). These costs may be increased during environmental change and possibly increase the risk of population extinction (Tanaka, 1996; Martínez-Ruiz and Knell, 2016) and there is some empirical evidence that sexual selection can increase extinction risk (Sorci, Møller and Clobert, 1998; Bro-Jørgensen, 2014). Additionally, environmental change may disrupt and alter mechanisms of sexual selection (Candolin, Salesto, & Evers, 2007; Evans & Gustafsson, 2017; Scordato, Bontrager, & Price, 2012; Tomkins, Hazel, Penrose, Radwan, & Lebas, 2011), potentially leading to changes in reproductive skew within populations (Järvenpää and Lindström, 2004). If the reproductive success of less well adapted individuals is increased due to environmental change and mechanisms of sexual selection disrupted, it is possible that the beneficial effects of sexual selection to population fitness may become reduced.

Due to the condition dependence of sexually selected traits it is likely that their expression may be reduced within altered environments (Kokko and Brooks, 2003). The condition dependence of sexually selected traits are often assessed by reducing resource availability, and there is evidence from a wide variety of taxa that individuals with reduced resources have decreased expression of sexually selected traits, strongly suggesting that these traits are condition dependent (Ohlsson *et al.*, 2002; Knell and Simmons, 2010; Rahman *et al.*, 2014;

Vega-Trejo, Jennions and Head, 2016). Environmental change is likely to have a similar effect. If individuals are moved from their optimal environmental conditions then the expression of sexually selected traits may become reduced. For example, mane characteristics in lions, *Panthera leo*, used in sexual signalling respond plastically and become reduced as ambient temperatures increase, probably as a likely consequence of altered costs of expressing darker longer manes at increased temperature (West and Packer, 2002). Furthermore, there is evidence from experimental populations of *Drosophila melanogaster* that males competing in an environment to which they are not adapted, have decreased mating success compared to adapted males, suggesting that the expression of courtship behaviours is based on condition and reduced within an altered environment (Dolgin, Whitlock and Agrawal, 2006).

Manipulations of mating systems through experimental evolution techniques have been used to investigate the outcome of differing intensities of sexual selection on sexually selected traits (Wilkinson and Reillo, 1994; Hosken and Ward, 2001; Pitnick *et al.*, 2001; Ingleby, Lewis and Wedell, 2010; Almbro and Simmons, 2014). It is expected that sexually selected traits will become more exaggerated under strong sexual selection and conversely reduced if sexual selection is weak. For example, within populations where males are exposed to high risk and intensity of sperm competition selection favours larger testes, increased sperm production and ejaculate size (Hosken and Ward, 2001; Pitnick *et al.*, 2001; Ingleby, Lewis and Wedell, 2010). Pre-copulatory sexually selected traits have also been shown to respond to artificial (experimenter assigned) selection, suggesting the potential for evolutionary change (Wilkinson and Reillo, 1994). Furthermore, male courtship rates (Holland and Rice, 1999) and female remating rate (Pitnick, Brown and Miller, 2001) have been shown to become reduced when evolving within populations in which sexual selection was removed by enforcing monogamy. Despite this, there is a surprising lack of evidence that mean values of

male pre-copulatory traits known to be under selection through female choice are increased by experimental evolution when selection is generated by female choice within a population (Hall, Lindholm and Brooks, 2004; McGuigan, Van Homrigh and Blows, 2008). A possible explanation for this is that male-male competition reduces the opportunity for female choice in a population.

Selection can act on individuals in a number of ways and in many species sexual selection will occur both pre- and post-copulation. Furthermore, multiple bouts of pre- and post-copulation selection are likely to occur within an individual's life. Understanding how different aspects of sexual selection act in relation to each other may provide further understanding in patterns of selection (Hunt *et al.*, 2009). There are examples of both multiple bouts or differing aspects of sexual selection having positive (Bangham, Chapman, & Partridge, 2002; Evans, Zane, Francescato, & Pilastro, 2003; Hosken *et al.*, 2008; House & Simmons, 2006; Lewis & Austad, 1994; Lewis *et al.*, 2013) and negative associations (Bernasconi and Keller, 2001; Danielsson, 2001; Preston *et al.*, 2001; Demary and Lewis, 2007), which are likely to increase and decrease variance in reproductive success, respectively. Positive associations suggest that in some cases individuals are overall superior competitors in good condition compared to other individuals in poor condition. Whereas, the observed negative associations may be caused by limitation of resources which males invest differentially into traits. In the latter case, it is likely that those traits under stronger selection will be exaggerated, possibly at the expense of traits.

Previous experimental work has shown that sexual selection can increase adaptation rates to environmental change (Fricke and Arnqvist, 2007; Plesnar-Bielak *et al.*, 2012; Jacomb, Marsh and Holman, 2016) and that environmental change can influence the expression of sexually selected traits and outcomes of sexual selection (Dolgin, Whitlock and Agrawal, 2006; Candolin, Salesto and Evers, 2007; Tomkins *et al.*, 2011). However, there is currently

no study which simultaneously investigates both the role of sexual selection in adaptation and the effect of environmental change on aspects of sexual behaviours and expression of sexually selected traits within the same experimental populations. This is a potentially important question to address as understanding which aspects of sexual selection are maintained or reduced during environmental change may provide insights into the expression of sexually selected traits but also what aspect of sexual selection is driving adaptive evolution.

Here, I present data estimating the expression of male pre- and post-copulatory sexually selected traits in the Indian meal moth, *Plodia interpunctella*, from populations in which the strength of sexual selection was manipulated in two experiments exposing populations to stable and increasing temperatures. *P. interpunctella* are a pest species living on stored food products with a polygamous scramble competition mating system from which female choice and sperm competition are likely to be under strong selection. There is evidence that males evolving with increased strength of sexual selection invest more in overall ejaculate, increasing both apyrene (non-fertile) and eupyrene sperm (fertile), respectively (Ingleby, Lewis and Wedell, 2010). The precise function of apyrene sperm is largely unknown but there is evidence it may have an important role in sperm competition and act to delay female remating (Cook & Wedell, 1999). Both sperm types have been shown to be reduced by poor nutrition suggesting condition dependence in overall ejaculate (Gage and Cook, 1994). Although not well described in the literature there appears to be good opportunity for females to choose their mates. Males perform a courtship display rapidly fanning their wings producing ultrasonic pulses (Trematerra and Pavan, 1995), which is possibly used by females to assess male quality (based on personal observations of courtship and mating). Notably, in another species of Lepidoptera, *Achroia grisella*, ‘song’ has been shown to be condition dependent (Brandt and Greenfield, 2004). Importantly males are unable to force copulations

and females accept matings from males by raising their abdomen for the male to engage with (personal observation).

To estimate pre-copulatory sexual selection mating assays which measured male mating success and latency to seduce females were conducted and this provided information on male attractiveness and condition. To estimate post-copulatory sexual selection testes dissections were performed and controlled for using male body size. Testes size is a common measurement made to estimate investment in sperm production and often used to quantify males sperm competitiveness (Hosken, 1997; Pitnick *et al.*, 2001; Byrne, Roberts and Simmons, 2002; Vahed and Parker, 2012). These data presented here were collected from the same male biased (MB) and female biased (FB) populations and at the same time data used as estimates of overall population fitness in chapter 2. Finding that offspring survival and fecundity under increasing temperatures was increased by evolving with strong sexual selection compared to weak sexual selection, suggesting that sexual selection was acting to promote the spread of good genes and increase adaptation rates. In contrast, there was no consistent effect of sexual selection under stable temperatures (see chapter 2). It is expected that the strength of both pre- and post-copulatory sexual selection will be altered by the manipulation of the adult sex ratio of populations. Traits associated with pre- and post-copulatory sexual selection are predicted to respond to an overall increase or decrease in sexual selection. Understanding which traits respond and if this relationship differs after environmental change may provide insight into which aspects of sexual selection are under strong selection, influence population fitness and enhance adaptation.

The increased opportunity for female choice in male biased populations is likely to filter male mating success based upon male condition, in comparison, in female biased populations females are expected to have little choice and accept matings from any male. Therefore, I predict to observe an overall increase in the mating success of males from populations

evolving with strong sexual selection compared to those populations with weak sexual selection. Furthermore, when competition is high (i.e. in MB populations) males which seduce females quickly are likely to experience increased reproductive success and therefore I predict to observe a decrease in the mating latency of MB males compared to FB males. Similarly, the increased risk and intensity of sperm competition in MB populations compared to FB populations is expected to increase the reproductive success of males with larger and more competitive ejaculates, I therefore predict to observe an increase in relative testes size of MB males in comparison to FB males. Due to the increased population fitness associated with strong sexual selection (MB) under increasing temperatures and the lack of difference in population fitness between MB and FB populations under stable temperatures (chapter 2), I expect the skew of reproductive success between MB and FB males to be greater under increasing temperatures. I therefore predict to observe a more pronounced difference between MB and FB males mating success and relative testes size under increasing temperatures compared to stable temperatures.

3.3 Methods

3.3.1 *Animal husbandry and experimental populations*

For detailed methods of animal husbandry and experimental evolution protocols see Chapter 2. In brief, sixteen replicate populations were established from a large outbred stock population of *P. interpunctella*. Experimental evolution populations were assigned to a treatment with differing intensities of sexual selection having either a male biased (MB) or female biased (FB) adult sex ratio, increasing or decreasing the strength of sexual selection, respectively. These experimental populations were then assigned to either the stable temperature (27°C) or to the increasing temperature (+2°C every other generation) experiments. Experiments ended once all populations from the increasing temperature experiment became extinct. In both experiments, male pre- and post-copulatory traits were assessed in the same way, starting from generation F₃. All adults used were virgin and less than 24hr old.

3.3.2 *Fitness assays*

3.3.2.2 *Pre-copulatory sexual selection*

Male pre-copulatory sexual selection was assessed through mating assays. In order to remove any time effects of mating behaviour all mating assays were performed 4 hours (\pm 30 minutes) after the start of the photophase. All mating assays were carried out in a controlled temperature room at 27°C and all moths given approximately 1 hour to acclimatise to vials at 27°C before mating assays began. Randomly selected males from experimental evolution populations were introduced to vials housing individual random females from the outbred stock population. Multiple mating assays were performed simultaneously by recording the time of male introduction and scanning all vials continuously noting the time of any

copulation. Mating assays lasted a maximum of 1 hour and if males failed to mate within 1 hour they were recorded as unsuccessful. This method provides two measurements of male ‘attractiveness’: whether a male was successful or unsuccessful in seducing a female and if successful the latent period prior to mating. Preliminary experiments performed during photophase and scotophase lasting 4 hours suggested that if males did not mate within 1 hour they were unlikely to mate after this time and no detectable behavioural differences were observed.

3.3.2.2 *Post-copulatory sexual selection*

Male post-copulatory sexual selection was assessed through testes dissections. Males were frozen and dissections performed at a later date, the amount of time males were kept frozen before dissections was kept as short as logistically possible. Before testes dissections were performed, the right forewing was carefully removed and kept frozen for later measurements (see below). The testes in *P. interpunctella* are fused and easily identified during dissections as a single, roughly spherical mass, with red pigmentation. Testes were dissected under a dissection microscope into a drop of PBS (phosphate buffered saline) and placed onto a microscope slide. The testes were relatively fragile and so were suspended in 30µl of PBS on a microscope slide for photographing. Photographs were taken using a camera mounted onto a microscope. Testes size was determined by importing images of testes into ImageJ and testes area measured by carefully drawing around the outside of the testes.

The right forewing of each male was used as a measure of body size (Lewis, 2005). Wings were dipped in succession into 90% isopropanol, 10% hydrochloric acid and commercial bleach before being rinsed in deionised water. This treatment removed scales and made veins in the wing visible (Reid, 1976). Wings were then mounted flat on microscope slides and allowed to dry for approximately 24 hours and photographed using a camera mounted onto a

microscope. The length between the junction of vein 1 and the point of insertion was measured using imageJ and used as an estimate of body size. A repeatability test was performed on 25 individuals that were randomly selected and repeatability was in high in both measurements (wing vein: $r = 0.95$; testes: $r = 0.99$).

3.3.3 Statistical analysis

All statistical analysis was performed using R statistical software version 3.3.0 (R Development Core Team, 2016). Model checks were performed to check for heteroscedasity of residuals and if necessary log transformation of response variables performed to improve model fitting. Generalised linear mixed models with binomial error structure were fitted to mating success data from both stable and increasing temperature experiments using the R package *lme4* (Bates *et al.*, 2015). General linear mixed models were fitted using *lme4* to log transformed mating latency and testes size data from both temperature experiments. In all models population was included as a random effect to account for repeated measures. The explanatory variables used in all models were sexual selection treatment and generation as a factor, including their two-way interaction term. The general linear mixed models fitted to testes size also included wing vein length as a fixed effect to control for body size.

3.4 Results

3.4.1 Mating success

In the increasing temperature experiment, 53.7% of 1112 males successfully gained copulations. A significant two-way interaction between sex ratio and generation was retained in the minimal adequate model ($\chi^2 = 11.37$; $df = 1$; $p = 0.045$; Figure 3.1 top panel; Table 3.1) indicating that male mating success differs between sex ratio treatments in certain generations. Inspection of figure 3.1 (top panel) shows that this interaction is largely driven by increased mating success of MB males in generation F_4 compared to FB males and that in all other generations mating success does not differ between sex ratio treatments. As temperature increases there is a reduction in male mating success but this is only statistically significant once the temperature males were reared at reached 33°C in generations F_7 and F_8 .

In the stable temperature experiment 58.3% of 1241 males successfully gained copulations. The two way interaction between sex ratio and generation was non-significant and was removed from the model ($\chi^2 = 6.87$; $df = 1$; $p = 0.23$). The main effects of sex ratio and generation were retained in the minimal adequate model. Overall, males from the MB treatment had an increased mating success compared to males from the FB treatment ($\chi^2 = 6.09$; $df = 1$; $p = 0.014$; Figure 3.1 bottom panel; Table 3.2). On average 62.9% ($\pm 4\%$) of MB males successfully gained copulations, compared to 49.9% ($\pm 5.4\%$) of FB males. The significant main effect of generation indicates that in generation F_4 there was an overall increase in mating success, whereas in generation F_6 there was an overall decrease in mating success of males from both MB and FB treatments ($\chi^2 = 20.9$; $df = 1$; $p < 0.001$).

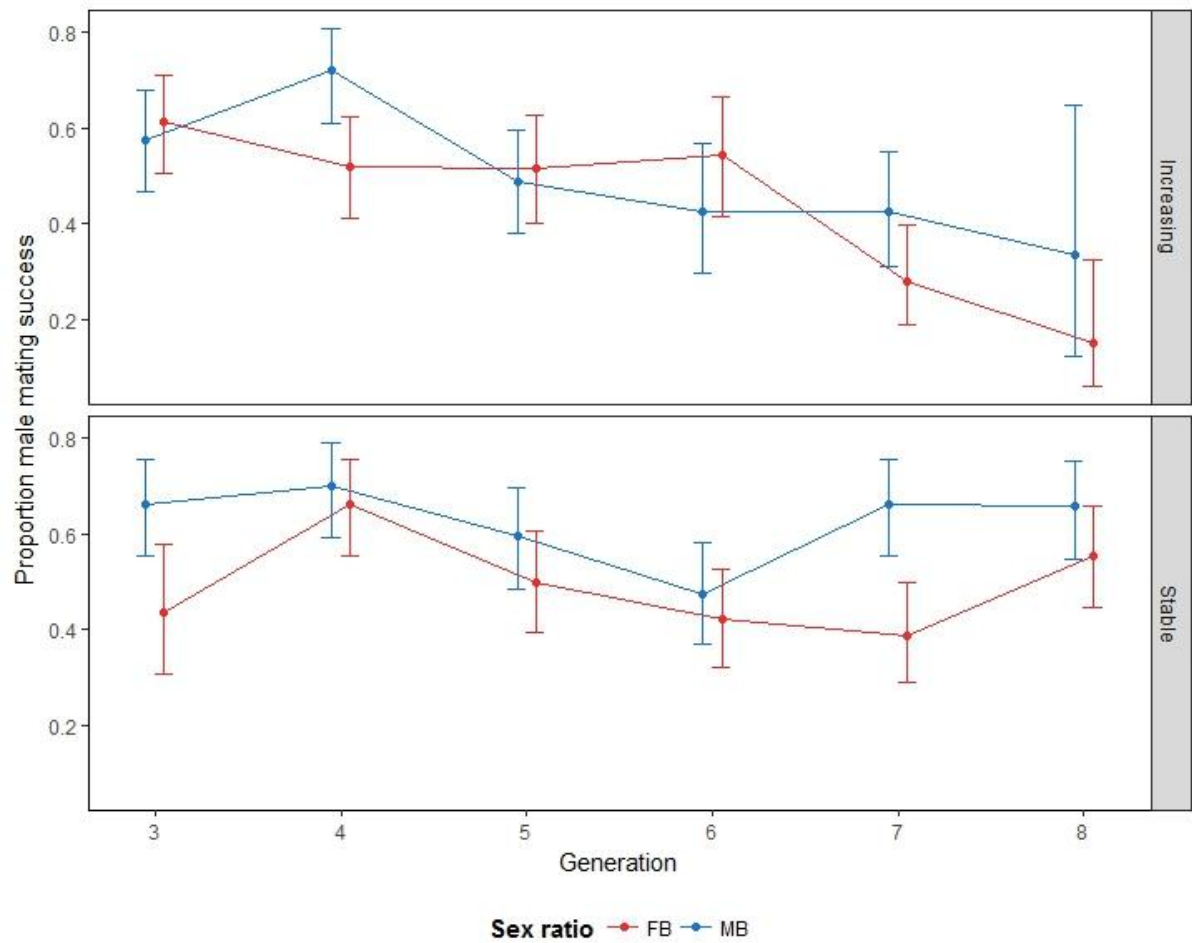


Figure 3.1: Proportion of males gaining successful copulations from male (MB: blue) and female biased (FB: red) populations from increasing (top panel) and stable (bottom panel) temperature experiments from generations F_3 - F_8 . All mating assays were carried out at 27°C .

Table 3.1: Results from fitting mixed effects model to predict male mating success, mating latency of successful males and relative testes size (body size estimated from wing length) of males in the increasing temperature experiment from generations F₃₋₈. The asterisk * indicates an interaction term between factors.

Source of variation	Male mating success		
	χ^2	df	<i>p</i>
Generation (as factor)	-	-	-
Sexual selection	-	-	-
Sexual selection * Generation (as factor)	11.37	1	0.045

Source of variation	Mating latency		
	χ^2	df	<i>p</i>
Generation (as factor)	-	-	-
Sexual selection	-	-	-
Sexual selection * Generation (as factor)	19.84	1	0.001

Source of variation	Testes size		
	χ^2	df	<i>p</i>
Wing length	48.28	1	< 0.001
Generation (as factor)	-	-	-
Sexual selection	-	-	-
Sexual selection * Generation (as factor)	24.7	1	< 0.001

Table 3.2: Results from fitting mixed effects model to predict male mating success, mating latency of successful males and relative testes size (body size estimated from wing length) of males in the stable temperature experiment from generations F₃₋₈. The asterisk * indicates an interaction term between factors.

Source of variation	Male mating success		
	χ^2	df	<i>p</i>
Generation (as factor)	20.9	1	< 0.001
Sexual selection	6.09	1	0.014
Sexual selection * Generation (as factor)	6.87	1	0.23

Source of variation	Mating latency		
	χ^2	df	<i>p</i>
Generation (as factor)	18.59	1	0.002
Sexual selection	1.67	1	0.197
Sexual selection * Generation (as factor)	6.03	1	0.303

Source of variation	Testes size		
	χ^2	df	<i>p</i>
Wing length	72.51	1	< 0.001
Generation (as factor)	-	-	-
Sexual selection	-	-	-
Sexual selection * Generation (as factor)	22.8	1	< 0.001

3.4.2 Mating latency

The model of mating latency of males from the increasing temperature experiment retained the two-way interaction between sex ratio and generation ($\chi^2 = 19.84$; $df = 1$; $p = 0.001$; Figure 3.2 top panel; Table 3.1). This interaction is driven by an increased mating latency of MB males in generation F_6 only and in all other generations there is no significant difference between the mating latency of males from different sexual selection treatments. Overall, being reared at increased temperature does not appear to influence the latency of males to mate.

In stable temperatures there was no difference in the mating latency of males from MB and FB sex ratio treatments indicated by a non-significant interaction between sex ratio treatment and generation ($\chi^2 = 6.03$; $df = 1$; $p = 0.303$) and non-significant main effect of sex ratio treatment ($\chi^2 = 1.67$; $df = 1$; $p = 0.197$). The main effect of generation was significant ($\chi^2 = 18.59$; $df = 1$; $p = 0.002$; Figure 3.2 bottom panel; Table 3.2) and indicates that in generations F_4 and F_7 both MB and FB males had reduced mating latency.

3.4.3 Testes size

In both experiments wing length, the estimate of body size, was a significant predictor of testes size with larger males having larger testes (increasing: $\chi^2 = 48.28$; $df = 1$; $p < 0.001$; stable: $\chi^2 = 72.51$; $df = 1$; $p < 0.001$). In the increasing temperature experiment there was a significant two-way interaction between generation and sex ratio treatment retained in the minimal adequate model ($\chi^2 = 24.7$; $df = 1$; $p < 0.001$; Figure 3 top panel; Table 3.1). This significant two-way interaction indicates that in generations F_3 , F_4 and F_6 MB moths have relatively larger testes, whereas in generations F_5 , F_7 and F_8 FB moths have relatively larger testes. Similarly the two-way interaction was retained in the model analysing testes size in the stable temperature experiment ($\chi^2 = 22.8$; $df = 1$; $p < 0.001$; Figure 3 bottom panel; Table 3.2).

This interaction is largely driven by MB males having increased relative testes size in generation F₅ but decreased relative testes size in generation F₇ compared to FB males. In all other generations there was no difference between males from the different sex ratio treatments.

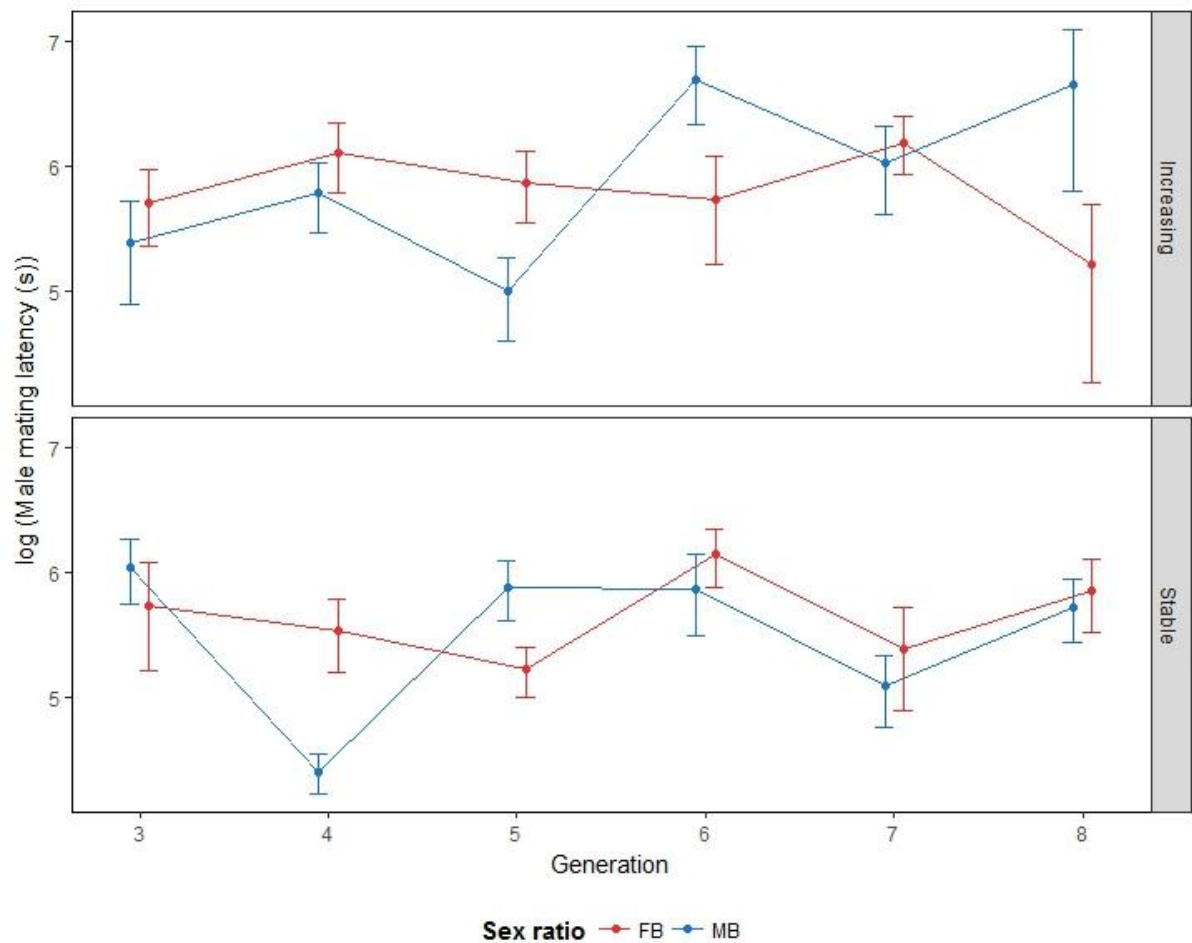


Figure 3.2: Differences in the log transformed mating latency of males which successfully gained copulations from male (MB: blue) and female biased (FB: red) populations from increasing (top panel) and stable (bottom panel) temperature experiments from generations F₃ - F₈. All mating assays were carried out at 27°C.

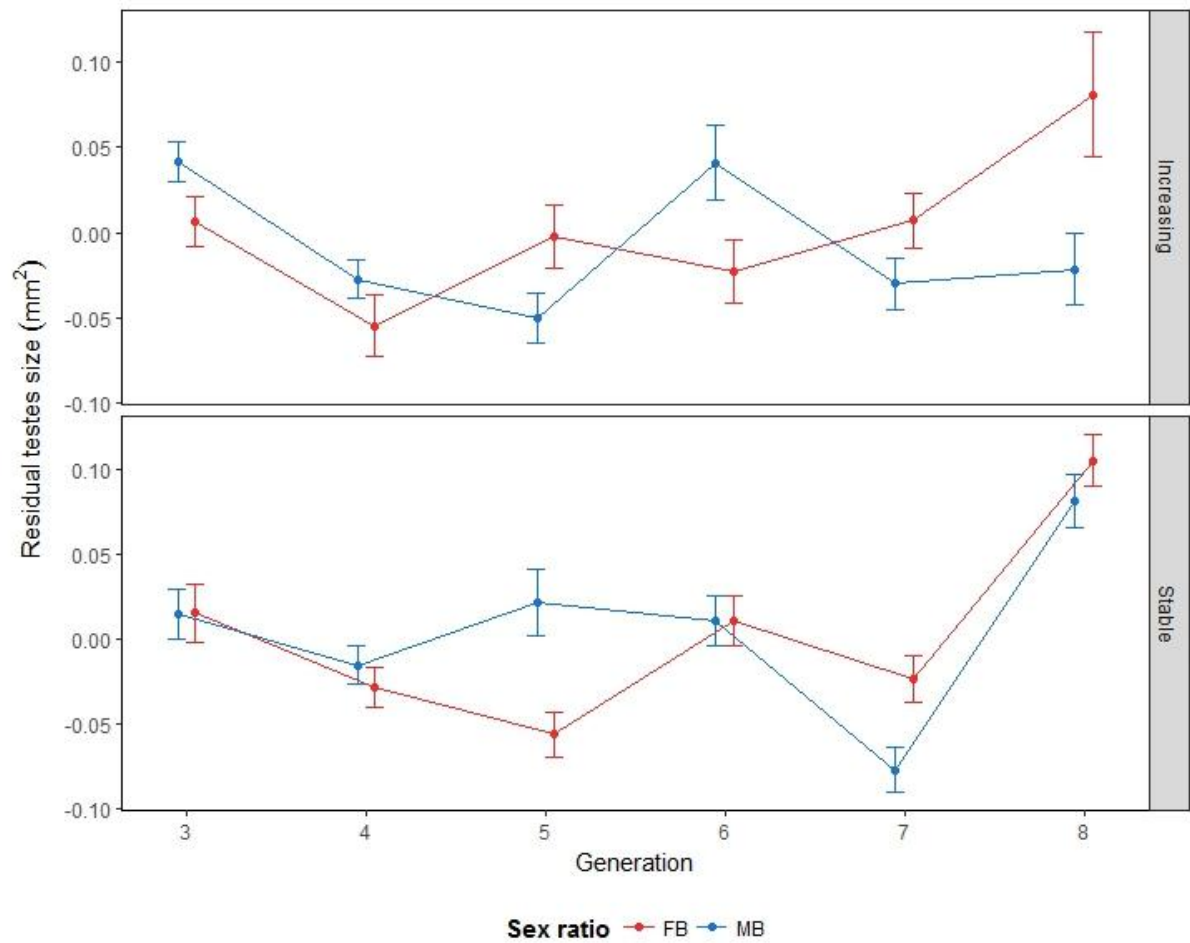


Figure 3.3: Differences in the residual testes size (after the removal of effects of body size) of males from male (MB: blue) and female biased (FB: red) populations from increasing (top panel) and stable (bottom panel) temperature experiments from generations F_3 - F_8 .

3.5 Discussion

It was predicted that both pre- and post-copulatory sexually selected traits would respond to an overall change in the intensity of sexual selection experienced by a population and that these may be altered by environmental change. Consistent with this prediction, male pre-copulatory sexual traits responded to the sexual selection treatment under stable temperatures, with an overall increase in the mating success of males from populations evolving with strong sexual selection compared to males from populations evolving with weak sexual selection. In contrast and against predictions, within the increasing temperature experiment no difference was observed between the mating success of males evolving with strong and weak sexual selection in the latter generations of the experiment. However, when temperatures were increased by only 2°C and after a generation within the increased temperature MB males have increased mating success compared to FB males, possibly suggesting that MB populations were more adapted to the slight increase in temperature. Furthermore, it was predicted that MB males would have a reduced mating latency compared FB males: contrary to this there was no general pattern and largely no difference in the mating latency between sexual selection treatments existed. Testes size differed between generations and this effect varied between the sex ratio treatments in both experiments. There was not a consistent pattern across both experiments, suggesting that the significant interaction terms reflect within generation variation rather than an evolutionary response. These findings are contrary to predictions and overall MB males did not evolve relatively large testes compared to FB males. Overall these results suggest that pre-copulatory sexually selected traits are under stronger selection in populations with increased sexual selection than post-copulatory sexually selected traits in this system. Furthermore, when temperatures are increasing the directional selection on male mating ability appears to be relaxed.

Male pre-copulatory mating success is likely to be largely based upon a set of behaviours performed during courtship displays in which males rapidly fan their wings and produce ultrasonic pulses (Trematerra and Pavan, 1995). Similar mechanisms have been described in the wax moth, *Achroia grisella*, in which males produce an ultrasonic ‘song’ within leks to attract females. Female choice favours certain song characteristics (Jang and Greenfield, 1996), and males vary in their song attractiveness (Jang and Greenfield, 1998) and evidence suggests that the song in *A. grisella* is condition dependent (Brandt and Greenfield, 2004). Moreover, the courtship displays of a variety of species have been shown to be condition dependent and honest signals of quality (Mappes *et al.*, 1996; Kotiaho, 2002; Pellitteri-Rosa *et al.*, 2011; but also see Gray & Eckhardt, 2001). It is likely that in *P. interpunctella* male song will also provide honest signals to females of male quality, possibly through similar song characteristics such as pulse rate, peak amplitude and gaps in song described in *A. grisella* (Jang and Greenfield, 1996) and could be an interesting avenue for future research to explore.

The species differ in how pair formation is facilitated, in *P. interpunctella* females advertise their position through olfactory signals (Brady *et al.*, 1971; Zhu *et al.*, 1999), whereas in *A. grisella* males attract females to their location through song (Spangler, Hayward, Greenfield and Takessian, 1984). This difference in mate location mechanisms is likely the reason to explain the lower frequency and amplitude of songs in *P. interpunctella* in which the signal will only need to be transmitted a short distance (Nakano *et al.*, 2009) compared to the metres in *A. grisella* (Spangler, Hayward, Greenfield and Takessian, 1984). Although possibly representing reduced costs of singing over a short distance, there is no reason to believe that a song of reduced frequency and amplitude would be any less effective as a signal of genetic quality within a species. Additionally these differences in mate location impose different

costs to male *P. interpunctella*, such as increased predation risk and mate detection in the scramble competition of males to gain matings.

The influence of strong and weak sexual selection within a population is likely to also cause responses in female pre-copulatory behaviours. Female choice is unlikely to be passive and by manipulating sex ratios the opportunity for female choice is altered, with less and more opportunity for choice within the FB and MB populations in the current study, respectively. Responses may be plastic but over multiple generations evolutionary change in female choosiness and mating rate may occur (Pitnick, Brown and Miller, 2001). Furthermore, there is some evidence that female choice may be dependent upon female condition (Hunt, Brooks and Jennions, 2005; Dakin and Montgomerie, 2014), which may be altered by increasing temperature. In order to remove any plastic or evolutionary changes of female choice which may occur within experimental populations, standardised females from stock populations were used during mating assays. Therefore any differences in mating success can be attributed to differences between experimental evolution population males.

With the increased opportunity for female choice in MB populations and the likelihood that female choice will be based upon the condition of males, when competition is strong, only those males in good condition will successfully gain copulations. In line with predictions, within the stable temperature experiment a significant main effect of sexual selection indicates that only two generations of experimental evolution and increased female choice were needed to alter the average mating success of males between sexual selection treatments. However, this overall increase in male mating success did not translate to increased population fitness (chapter 2). Suggesting that increased male mating success does not necessarily translate to an overall increase in population fitness, potentially caused by the intra-locus sexual conflict known to occur within *P. interpunctella* (Lewis et al., 2011).

Whereby, directional selection on male reproductive success may have a negative effect on

female fitness as genes related to high fitness in males when expressed in females could potentially reduce female specific fitness (Chippindale, Gibson and Rice, 2001) and negate fitness benefits caused by sexual selection.

In contrast, at increasing temperatures MB males only had increased mating success after one generation when the temperature was raised by 2°C, however once the temperature was increased further this difference between sexual selection treatments disappeared. This result indicates that the expression of male pre-copulatory sexual traits may be altered by being reared at changing temperatures; however the direction is opposite to the predicted pattern and variance in male mating success was reduced under increasing temperatures. Under directional natural selection the benefits of sexual selection are expected to be increased because variance in reproductive fitness is based upon adaptive and maladaptive alleles (Long, Agrawal and Rowe, 2012) and the results from chapter 2 are in agreement with this. Against predictions the increased population fitness did not also translate to an overall increase in male mating success, and the observed increase mating success of MB males found under stable temperatures was not paralleled under increasing temperatures.

Variance in male mating success may not be captured using this method of mating assay. Mating assays were staged in vials with individual males and females, this artificial staging may not capture what is occurring within experimental populations in which females are able to make their mate choice from multiple males simultaneously. It would be interesting to test this by placing multiple marked males from different sexual selection treatments with a single female and observing male mating success under competition. Furthermore, these staged mating assays also only capture a single mating opportunity whereas in population pots males have the potential to mate with multiple females which may be both virgin and non-virgin. It has previously been shown that males who successfully mate with non-virgin females have an increased number of lifetime matings in *P. interpunctella* (Lewis et al., 2013) and that

males from male biased populations successfully gain a greater number of lifetime matings than males from female biased populations (Willis, 2015). It is possible that the mating assays in the current study do not capture the variance in male lifetime reproductive success in non-adapted populations (i.e. increasing temperatures) and that it is number of matings and ability of males to seduce non-virgin females which is driving the observed increase in adaptation rates (chapter 2). Addressing this would be insightful and an area for future research to address.

A potential limitation of this study is that mating assays were performed at the same temperature, regardless of the rearing temperature. This may explain the lack of difference between sexual selection treatments in mating success when temperatures were increased by 4°C or more. Mating success has been shown to be reduced when competing in an environment to which males are not adapted (Dolgin, Whitlock and Agrawal, 2006). However, the general lack of difference between generations in the mating latency of males from increased temperatures suggests that within the current study, male behaviour was largely unchanged by these differences in rearing and mating assay temperatures. The increasing temperature experiment was performed this way for two reasons. Firstly the stock population was reared at the ancestral temperature (27°C), and if mating assays carried out at increased temperatures the inverse effect of females being at higher temperatures than what they were reared at would occur. An increasing temperature stock population could have been made alongside the increasing temperature experiment and mating assays performed at increased temperatures. However, and the second reason, due to our experimental setup it would have made it near impossible to observe mating events within an incubator at increased temperatures.

An alternative explanation may also exist to explain the observed increase in MB males mating success compared to FB males at stable temperatures. Mating imposes costs to males

and males are likely to invest resources (i.e. ejaculate) adaptively (Wedell, Gage and Parker, 2002). *P. interpunctella* males are known to be able to adjust their investment in ejaculate based upon female size and therefore reproductive potential (Gage, 1998), and generally there is widespread evidence males respond adaptively to differences in female mating status and quality (Schwagmeyer and Parker, 1990; Bonduriansky, 2001; Wong and McCarthy, 2009; Ala-Honkola *et al.*, 2015; Chenoweth *et al.*, 2015). Due to differing investment of ejaculate or male mating preferences, FB females may be higher quality due to increased selection imposed by males compared to MB females. Therefore the observation of differences in mating between sexual selection treatments in the stable temperature experiment may be caused by FB males being less willing to copulate with stock population females. Again though, the general lack of observed difference in mating latency between sexual selection treatments and that the vast majority of males attempted to copulate with females (personal observation) suggests that male willingness to mate with stock population females is an unlikely explanation, however this cannot be entirely ruled out.

In both experiments testes size appears to be extremely variable between generations and contrary to predictions there do not appear to be overall patterns or differences in male investment in testes after controlling for body size between sexual selection treatments. However, there is a suggestion that under increasing temperatures FB males are investing more in testes than MB males toward the latter half of the experiment when temperatures are increased. This is counter to what was predicted, where increased risk and intensity of sperm competition should generally favour increased investment in testes and ejaculate (Hosken and Ward, 2001; Ingleby, Lewis and Wedell, 2010) and appears to be a consequence of large variation between generations rather than evolutionary change. Testes mass has previously been found to have substantial variation between generations in *Onthophagus taurus* (Simmons and Garcia-Gonzalez, 2008; Almbro and Simmons, 2014), and has been attributed

the condition dependence of testes size and to possible differences in quality of diet between generations (Almbro and Simmons, 2014). This explanation seems unlikely within the current study and why there is so much variation in testes size between generations is difficult to explain. Interestingly in both Almbro & Simmons (2014) and the current experiment it is those populations moved from their adaptive peaks through induced mutation or environmental change, respectively, that have the greatest amount of variation between generations.

After over 55 generations of experimental evolution at differing risks and intensities of sperm competition the number of sperm within *P. interpunctella* ejaculates show clear patterns of divergence and it seems that increased sperm competition favours increased quantity of sperm (Ingleby, Lewis and Wedell, 2010). It is possible that the current study was not long enough to see similar divergence in testes size. However, only relatively few generations of experimental evolution between monogamous and polyandrous populations of yellow dung flies, *Scathophaga stercoraria*, were needed to see differences in testes size (Hosken and Ward, 2001). When sexual selection was allowed or removed from populations of *O. taurus* with induced mutations it was only male strength, a pre-copulatory trait, and not testes mass that responded, suggesting weaker selection on post-copulatory traits compared to pre-copulatory traits. Results from the stable temperature experiment are consistent with this suggestion.

Overall these results provide evidence that under stabilising selection, strong sexual selection can increase the average mating success of males, suggesting that female choice is skewing reproductive success in favour of males with specific traits inherited by their sons and possibly reflect overall condition. Taken with previous results (chapter 2) this suggests that male mating success does not translate to increased population fitness, possibly due to sexual conflict. When populations were reared under directional selection this pattern of increased

male mating success largely disappears, despite this apparent lack of selection through female choice, those populations under strong selection appear to have an increased adaptation rates (chapter 2). As populations are moved from their adaptive peaks it is likely that variation in reproductive success will increase and it is possible under increasing temperatures that the single observation of male mating success in these mating assays does not capture the variance of male lifetime mating success. The difference between experiments may reflect changing costs and benefits of sexual selection under stabilising and directional natural selection.

Chapter 4: Does sexual selection influence the persistence and adaptation of dung beetle species after modification and conversion of tropical rainforest?

Dr Eleanor Slade carried out dung beetle collections in 2011. Dr Eleanor Slade and Sol Milne helped with dung beetle collections in 2015. Identification of dung beetles collected in 2011 was carried out by Dr Eleanor Slade, Darren Mann and myself. Advice on molecular work was given by Prof. Stephen Rossiter and Rosie Drinkwater.

4.1 Abstract

Understanding how species adapt to and persist within altered environments is a pressing goal within our rapidly changing world. A major cause of habitat change is caused by conversion and over use of resources in agriculture, most notably in tropical forest regions. Identifying which species may be more prone to extinction and those which are most able to persist within these altered habitats is of critical importance to conservation of biodiversity. Sexual selection is ubiquitous in sexually reproducing species to varying degrees but there is controversy and debate about its effects on population fitness, with previous findings suggesting sexual selection can increase extinction risk but also increase adaptation rates. In the present study dung beetle communities were surveyed across a gradient of habitat modification in Sabah, Malaysian Borneo, and proxies of the intensity of sexual selection experienced by species was estimated from morphological traits. There is a general trend of species found in old-growth forest to become less abundant or to disappear as the intensity of habitat modification increases. Horned species however, tend to persist and maintain high abundances across the disturbance gradient, with strong evidence within low intensity logged areas. In contrast, hornless species show reduced abundance across the disturbance gradient and do not persist in the oil palm plantations at all. Furthermore, horned species with

increased intensity of male contest competition, estimated from possessing relatively large horns, are those species which maintain similar abundances across the habitat disturbance gradient and species with relatively small horns which on average show reduced abundance in disturbed habitats. These results suggest that male contest competition and the strength of this competition between males may drive adaptation to novel environments and increase the likelihood of species persistence in altered habitats. A weak negative effect of testes mass on species abundance in the most disturbed sites was found and sexual size dimorphism appears to have no association with species abundance across all sites, suggesting that different sexually selected traits may differ in their costs and benefits with a degree of trait specificity. These results provide the first evidence from the field that sexual selection, in this case male-male competition, can increase adaptation and the persistence of species within novel and altered environments.

4.2 Introduction

Around the globe there is increasing anthropogenic environmental change. A major concern for world biodiversity is loss of forests caused by over exploitation or modification, with logging and agriculture expansion and intensification among some of the main causes (Phalan *et al.*, 2013). Within modified landscapes there are many environmental factors which are likely to change and possibly affect species persistence or abundance: for example, resources such as food or breeding sites may become limited (Lamanna and Martin, 2016) and abiotic factors such as temperature and humidity altered (Ashcroft and Gollan, 2012; Hardwick *et al.*, 2015). It is likely that species traits may affect the persistence of populations within altered habitats, with generation time, reproductive potential and phenotypic plasticity among a number of traits identified to likely influence a population's adaptive capacity and resilience to environmental change (Chevin, Lande and Mace, 2010). The strength of sexual selection a species or population experiences has also been suggested to both have the potential to drive adaptive evolution but also possibly increase extinction risk (Candolin and Heuschele, 2008; Holman and Kokko, 2013). Sexual selection is ubiquitous in sexually reproducing species to varying degrees, therefore understanding any effects sexual selection may have on the vulnerability of populations facing environmental change is of clear importance.

Sexual selection can be defined by two main processes, male competition and female choice, which can both occur pre- and post-copulation. Pre-copulatory male contest competition, where males compete for access to copulations favours traits such as increased male body size and weaponry (Darwin, 1871; Andersson, 1994). Male contest may continue post-copulation if females mate with multiple males in sperm competition, where male fertilisation success is often proportional to numbers of competing sperm, selecting for increased

investment in spermatogenesis and testes size (Parker, 1970). Females may sample a number of males and base their mate choice upon specific traits, driving the evolution of elaborate and colourful male displays (Darwin, 1871; Andersson, 1994). Furthermore, this choice can occur post-copulation and females may be able to manipulate which male's sperm successfully fertilise their eggs, in a process named cryptic female choice (Eberhard, 1996). Multiple traits may be associated with reproductive success and differing aspects of sexual selection are likely to be acting simultaneously within populations.

Sexually selected traits are generally negatively associated with an individual's survival because of the inherent costs of growth, maintenance and conspicuousness (Andersson, 1994; Rowe and Houle, 1996). However, selection can favour the evolution of costly sexually selected traits because the bearers of the most exaggerated secondary sexual traits generally achieve increased reproductive success (Darwin, 1871). Furthermore, females often gain indirect genetic benefits because their offspring are sired by males of high genetic quality (Andersson, 1994; Jennions and Petrie, 2000). A proposed mechanism of this 'good genes' sexual selection is the genic capture model which has gained wide support as it provides a possible resolution to the lek paradox (Rowe and Houle, 1996; Kotiaho, Simmons and Tomkins, 2001; Tomkins *et al.*, 2004; Cotton, Small and Pomiankowski, 2006). The genic capture model postulates that the expression of sexually selected traits is condition dependent and that condition is based upon the overall genetic quality of the bearer (Rowe and Houle, 1996). These ideas have gained empirical support and good genes sexual selection has been proposed to potentially increase adaptation rates and decrease the extinction risk of populations (Fricke and Arnqvist, 2007; Jarzebowska and Radwan, 2010; Plesnar-Bielak *et al.*, 2012; Lumley *et al.*, 2015).

Sexual selection has been suggested to enhance the purging of deleterious alleles and promote beneficial alleles within populations (Whitlock, 2000; Agrawal, 2001; Siller, 2001;

Whitlock and Agrawal, 2009). These arguments are largely based upon competition or choice, leading to non-random mating and fertilisation success, which due to genic capture will be inversely proportional to mutation load (Tomkins *et al.*, 2004). Therefore males with above average reproductive success will have fewer deleterious alleles than the population average. Similarly, this has led researchers to propose that good genes sexual selection might also have implications for the adaptive capacity of populations during environmental change (Lorch *et al.*, 2003). Within an altered environment those individuals which are closest to the populations adaptive peak will have the greatest expression of sexually selected traits, skewing reproductive success in their favour and therefore the spread of beneficial alleles throughout the population (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016). Long *et al* (2012) suggested that when variation in condition dependant sexually selected traits is driven by maladaptive and adaptive alleles, natural and sexual selection are likely to align and increase the fitness of a population containing an influx of maladapted alleles.

There is empirical support for sexual selection increasing adaptation rates from laboratory evolution experiments. Populations of bulb mites *Rhizoglyphus robini* exposed to increased temperature (Plesnar-Bielak *et al.*, 2012), seed beetles *Callosobruchus maculatus* given a novel diet (Fricke and Arnqvist, 2007) and flour beetles *Tribolium castaneum* exposed to pesticide (Jacomb, Marsh and Holman, 2016), in which sexual selection was experimentally removed, had decreased adaptation rates compared to populations in which sexual selection was allowed to occur.

In contrast to sexual selection driving adaptation and increasing the average fitness of individuals within a population a number of comparative field studies indicate that the inherent costs of sexual selection can increase extinction risk or negate any benefits of sexual selection and have a neutral effect. Sexually dichromatic species of birds were less likely to persist when introduced to novel islands (McLain, Moulton and Redfearn, 1995; Sorci,

Møller and Clobert, 1998; McLain, Moulton and Sanderson, 1999) and to have increased probability of localised extinction across North America (Doherty *et al.*, 2003), compared to monochromatic species. Studies comparing a wider range of species and inferring extinction risk from IUCN conservation status tend to show no association between extinction risk and measures of sexual selection in birds and mammals (Morrow and Pitcher, 2003; Morrow and Fricke, 2004; Bro-Jørgensen, 2014). However, bovid species with relatively large horns (Bro-Jørgensen, 2014) and bird species with relatively large testes (Morrow and Pitcher, 2003) were found to be at greater risk of extinction, suggesting that the cost of pre- and post-copulatory sexual selection can increase extinction risk, respectively.

There are a number of factors that may contribute to sexual selection being detrimental to persistence and adaptation within novel environments. Inbreeding may increase due to the reproductive skew caused by sexual selection reducing the effective population size (Kokko and Brooks, 2003). The cost of bearing extravagant and exaggerated secondary sexual traits may trade-off against male investment in survival traits and increase male mortality (Promislow, 1992; Promislow, Montgomerie and Martin, 1992). Tanaka (1996) showed that during environmental change those populations experiencing sexual selection are theoretically more likely to go extinct because of this increased load. Furthermore, increased male mortality, caused by sexually selected traits, has been shown to make small sexually selected populations particularly prone to extinction due to increased risk of demographic stochasticity removing all males from populations (Martínez-Ruiz and Knell, 2016).

Additionally, as variance in reproductive success caused by sexual selection increases correspondingly, so does the opportunity for sexual conflict to occur. Inter- and intra-locus sexual conflict (Chapman *et al.*, 2003; Bonduriansky and Chenoweth, 2009) may decrease the adaptive capacity of populations and increase extinction risk, or negate any benefits of sexual selection and cause a neutral effect. These negative effects of sexual selection caused

by sexual conflict occur because traits which increase male reproductive fitness may act antagonistically to female fitness (Holland, 2002; Kokko and Brooks, 2003; Rundle, Chenoweth and Blows, 2006; Chenoweth *et al.*, 2015).

Mechanisms may exist, however, which minimize these costs of sexual selection. Inbreeding depression may be reduced by sexual selection (Jarzebowska and Radwan, 2010; Lumley *et al.*, 2015) and condition dependency may reduce the expression and overall costs of sexually selected traits when populations are stressed (Kokko and Brooks, 2003). Furthermore, intralocus sexual conflict may help to maintain genetic diversity (Foerster *et al.*, 2007) and interlocus sexual conflict may purge deleterious mutations through selection on female ability to maintain reproductive fitness (Wigby and Chapman, 2004; Michalczyk *et al.*, 2011).

There is contradicting evidence between laboratory and field studies (e.g. Sorci, Møller and Clobert, 1998; Plesnar-Bielak *et al.*, 2012), showing that sexual selection can potentially have both beneficial and negative effects to populations facing environmental change. Intra-specific laboratory studies, typically demonstrate sexual selection increases population fitness. However, this is achieved by manipulating the intensity of sexual selection through enforcing monogamy or allowing polyandry (Fricke and Arnqvist, 2007; Plesnar-Bielak *et al.*, 2012; Jacomb, Marsh and Holman, 2016). This manipulation of mating systems only provides evidence that the removal of sexual selection in species that are naturally polygamous is detrimental to a population facing environmental change. Comparative studies provide information into species prior evolutionary responses to sexual selection and how those traits favoured by sexual selection may influence adaptation and extinction risk (e.g. Sorci, Møller and Clobert, 1998; Morrow and Pitcher, 2003). When assessing the effect of sexual selection in terms of biodiversity and conservation management, comparative studies are likely to be more informative than intra-specific laboratory studies in identifying which species are more likely to be vulnerable to environmental change. Comparative studies have

suggested the negative effects sexual selection can have on populations may be exaggerated by anthropogenic driven environmental change (Morrow and Fricke, 2004; Bro-Jørgensen, 2014). However, the only comparative studies specifically investigating the effect of sexual selection in novel environments come from historical introductions of birds to novel islands (McLain, Moulton and Redfearn, 1995; Sorci, Møller and Clobert, 1998; McLain, Moulton and Sanderson, 1999). As suggested by Martínez-Ruiz & Knell (2016), it is possible that these results were caused by the relatively small populations that were introduced and findings may not be generalisable to larger populations.

Here, we assess the effect of sexual selection on the persistence of dung beetles species (Coleoptera: Scarabaeidae: Scarabaeinae) across a gradient of habitat modification from old-growth tropical rainforest to oil palm plantation in Sabah, Malaysian Borneo. Tropical forests contain much of the world's biodiversity and are ecosystems which are facing unprecedented rates of deforestation and habitat modification (Bradshaw, Sodhi and Brook, 2009), South East Asia in particular has some of the highest relative loss of forest and degradation (Sodhi *et al.*, 2004). Predicting how species and communities are affected and respond to modification of tropical forest is therefore a major concern for conservation ecologists. Dung beetle biodiversity and abundance have been shown to be altered by the impacts of logging and conversion of forest to agriculture, with a general pattern emerging with increasing habitat modification decreasing biodiversity and abundance (Braga, Korasaki, Andresen, & Louzada, 2013; Edwards *et al.*, 2013, 2017; Gray, Slade, Mann, & Lewis, 2014; Nichols *et al.*, 2007; Slade, Mann, & Lewis, 2011). We specifically investigate the effect that traits associated with sexual selection may have on the persistence and abundance of dung beetle species after the logging and modification of old growth forest.

Dung beetles are not only an ecologically important group (Nichols *et al.*, 2008), but also an ideal group to address this question as they exhibit many aspects of sexual selection. In dung

beetles, male contest competition has caused the evolution of a bewildering variety of horn structures of differing shapes and sizes (Emlen, 2008; Emlen et al., 2005). Increased horn lengths are generally associated with an increase in male competition (Andersson, 1994; Bro-Jørgensen, 2007). Furthermore, there is evidence that horn length-body size allometry can be non-linear (Knell, 2009a), often leading to distinct male morphs in which small ‘minor’ males express disproportionately small or no horns and large ‘major’ males express disproportionately large horns (Emlen, 1996; Emlen, Hunt, & Simmons, 2005; Moczek & Emlen, 2000). Differing allometry patterns may provide evidence of differing costs and benefits of the expression of weaponry within and between species (Emlen, 2001; Emlen & Nijhout, 2000; Knell, Pomfret, & Tomkins, 2004; Mccullough et al., 2015; Moczek, Brühl, & Krell, 2004; Pomfret & Knell, 2006). There is evidence both within and between species that the risk and intensity of sperm competition varies and accordingly males are known to invest differentially in testes (Simmons, Emlen and Tomkins, 2007; Simmons and García-González, 2008). Within a number of dung beetle species male courtship rate has been shown to be under female selection, suggesting female choice could also be a general aspect of sexual selection within dung beetles (Kotiaho, Simmons and Tomkins, 2001; Kotiaho, 2002). Importantly, horn length, strength, testes size and male courtship rate have been shown to be condition dependent in those species studied (Emlen, 1994; Knell & Simmons, 2010; Kotiaho, 2002; Kotiaho et al., 2001; Simmons & Garcia-Gonzalez, 2008; Simmons & Kotiaho, 2002)

We present biodiversity and abundance data of dung beetle communities within habitats which have been modified to varying degrees. We test for associations between traits used as surrogate indices of the strength of sexual selection experienced by a species and species persistence across the disturbance gradient. We investigate whether there is a difference in species richness and abundance between horned and hornless species. Within the horned

species, we ask whether relative horn length affects species abundances in altered habitats, and whether this is related to the various allometric patterns that we find. Furthermore, we investigate the relationship between sexual size dimorphism and relative testes mass with species abundance across the disturbance gradient.

Overall, we predict to observe a decrease in both species richness and abundance of species as the disturbance of habitat increases, with fewer species at lower abundances persisting across the habitat disturbance gradient. Sexually selected traits are expected to alter population fitness, and the costs and benefits of sexual selection may be altered by environmental change. We predict that if the costs of expressing horns in altered habitats increase we will observe a negative association between horned species and persistence across the disturbance gradient when compared to hornless species. Alternatively, if sexual selection, via male contest competition, skews reproductive success towards individuals most well adapted to the altered habitats we would expect to see an increased persistence of horned species across the disturbance gradient compared to hornless species. Similar predictions were made regarding relative horn lengths of horned species, if costs of expressing relatively large horns increase in altered habitats we expect to see reduced abundance of species with relatively large horns in disturbed habitats compared to species with relatively small horns. Alternatively, species with relatively large horns are expected to have increased intensity of competition and increased male reproductive skew, if this increased skew favours males that are most well adapted within altered habitats we expect to observe species with relatively large horns persisting in altered habitats at higher abundances compared to species with relatively small horns. Making explicit predictions is therefore difficult and our observation will likely be determined by the net cost and benefit of horn expression to a population within the altered habitats.

Whether the allometric relationship between horn length and body size will influence the persistence of species in altered habitats is equally difficult to predict partly because no similar investigations have been performed. The mechanisms which determine the reprogramming of major and minor beetles are thought to differ between discontinuous and continuous horn length-body size allometries. If these differences alter an individuals' ability to match their phenotype to environmental conditions we expect to observe a difference in the abundance of species with different allometric relationships between horn length and body size.

We predict to observe an association between sexual size dimorphism and species persistence in altered habitats. Species with increased intensity of male-male competition are expected to have relatively large males compared to females. If increased reproductive skew drives adaptation to altered habitats and we predict to observe species with relatively large males to persist across the disturbance gradient at higher abundances compared to species with relatively large females.

Relative testes size is assumed to be associated with the intensity and risk of sperm competition within a species; with larger testes being favoured in species with increased sperm competition. Larger testes are expected to be associated with increased levels of polyandry and hence genetic diversity of offspring. We therefore predict that those species with relatively large testes will have a greater ability to adapt to and maintain higher abundance in altered habitats compared to species with relatively small testes.

4.3 Methods

4.3.1 Study site and collections

Study sites were located within the Stability of Altered Forest Ecosystems (SAFE) project site (4°33'N, 117°16'E) and the Maliau Basin primary forest reserve (4°49'N, 116°54' E) in Sabah, Malaysian Borneo. The SAFE project is a large scale experiment investigating the effects of rainforest modification and fragmentation consisting of a mixture of twice logged lowland dipterocarp rainforest and oil palm plantations which were planted between 2000 and 2006 (Ewers *et al.*, 2011).

Dung beetle sampling was performed between June and August in 2011 and again between July and August in 2015. Sampling in 2011 was performed before experimental fragmentation began (described in Ewers *et al.*, 2011) and the habitats are classified as heavily logged fragmented forest (HLF) within the current study. Sampling of dung beetles was carried out across a gradient of forest modification: the Maliau Basin primary forest reserve was unaltered old growth forest (OGF); and disturbed sites were from within the SAFE landscape: twice selectively logged continuous forest (SLF), twice heavily logged fragmented forest (HLF) and oil palm plantation (OPP). Details in the differences in intensity of logging and forest cover between sites are provided in Ewers *et al.*, (2011) and details in a number of abiotic factors in Hardwick *et al.*, (2015).

The SAFE project experimental design has two different sampling networks for continuous (OGF, SLF and OPP) and fragmented (HLF, forest fragments in (Ewers *et al.*, 2011)) habitats. Sampling stations in continuous habitat (OGF, SLF and OPP) use hierarchical triangular fractal geometry at four spatial scales and the corners of each hierarchical triangle are used as a sampling station. The centre of each fourth order point is located 1780m from each other; from each of these points third order points are located 564m from each other; we

sampled from second order sampling stations which are centred on third order points and are located a minimum of 178m from each other (figure 1a,b,d). This spatial scaling is greater than the recommend minimum of 100m distance between traps used in dung beetle sampling (Da Silva and Hernández, 2015). In each continuous habitat type we sampled from three replicates (fourth order: i.e. OG1, OG2 and OG3 in figure 1a), with 9 sampling stations per replicate (second order: black circles in figure 1a,b,d; see Ewers *et al.*, (2011) for more details in sampling design). Sampling stations in fragmented habitat (HLF) were designed for once forest fragmentation occurred and the spatial scaling is different to that of continuous habitats, second order sampling stations at each replicate represents branching transects (figure 1e; see Ewers *et al.*, (2011) for more details in sampling design). Sampling took place from all 16 second order sampling stations (black circles in figure 1e) from three HLF replicates (Fragments B, D and E; see Figure 1e). In order to balance sample effort only nine traps from each HLF replicate were randomly selected and included in analysis.

In total, 108 traps were used to determine species richness and abundance data, 27 traps per habitat type with 9 traps in each of the 3 replicates. In 2015 trapping methods were similar to 2011, except in HLF only 9 traps were set at random second order sampling stations and in SLF only 3 traps were set per replicate. The latter was due to logistical reasons in which beetles used for testes dissections would have been starved for multiple days before processing began (see below).

In 2011 dung beetle collections primarily set out to assess species richness and abundance along the gradient of forest modification, whereas in 2015 dung beetle collections were performed primarily to gain measurements of testes mass. In both 2011 and 2015 sampling was carried out using pitfall traps baited with 25g of human dung protected from rain with a cover. Human dung is widely used as standardised bait in tropical forests (Marsh *et al.*, 2013) and attracts a wide variety of dung beetle species (Davis *et al.*, 2001; Larsen, Lopera and

Forsyth, 2006). Trapping methods differed slightly between years, in 2011 pitfall traps were half filled with a solution of water, salt and detergent which kills beetles as they break the water surface tension and cannot escape, and traps were left for 48hrs before being collected. In 2015 live pitfall trapping was carried out and used a plastic funnel to minimize beetle escapes, and traps were set and left for 24hrs before being collected. Therefore all data on biodiversity and species abundance is from 2011 only because comparisons cannot be made between years.

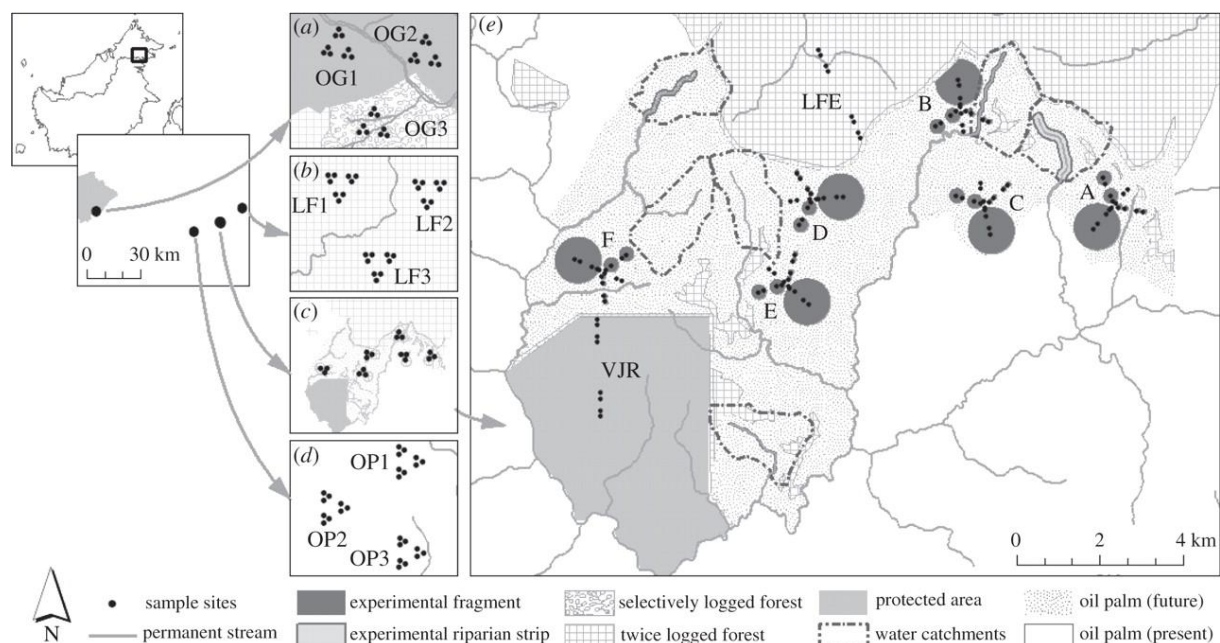


Figure 4.1: Map of the SAFE Project, located in Sabah, Malaysian Borneo (taken from Ewers *et al.*, 2011), showing the gradient of forest modification (a) old growth forest (OGF); (b) twice selectively logged continuous forest (SLF); (c&e) twice heavily logged fragmented forest (HLF) and (d) oil palm plantation (OPP). A Virgin Jungle Reserve (VJR) and the edge of continuous logged forest (LFE) are also shown.

Specimens from 2011 were stored in 90% ethanol and identified using the reference collections housed in the Oxford University Museum of Natural History (OUMNH). Specimens from 2015 were identified in the field. All identification was to the species level. In total, 57 species from 2011 and 37 species from 2015 were identified. The difference in the total number of species identified between years was possibly caused by the reduced amount of time traps were left out for in 2015 and/or because live trapping may be more prone to smaller species being able to escape or being predated from traps. Those species which were not re-caught in 2015 tended to be relatively small and also had relatively low abundances in 2011. To determine which species to include in the final dataset, a number of criteria were used (see below), and only 3 species in the final dataset were not trapped in 2015.

4.3.2 Trait measurement

Specimens from both 2011 and 2015 were photographed using a USB microscope, images were imported to imageJ and measurements made. Pronotum width was used as our measure of body size. For horned species all males were photographed from 2011 and in the most abundant species only a proportion of females were photographed because one of the main aims of this study was to investigate the effects of horn length and testes size. If species were horned male horns were photographed from two angles, front and side. A number of species also possessed pronotum horns and these were also photographed from two angles based upon the shape of horns. The average of the two measurements of head and pronotum horns were used, to gain a more reliable measurement of horn length between species and to minimize any potential bias of using a single measurement method because the shape and structure of dung beetle horns are extremely variable (Emlen et al., 2005). There was also variation in the number of horns between species, some having paired or single head and pronotum horns. A composite measurement of total horn investment was therefore calculated

by multiplying horn length by the number of horns of that type (if horns were paired only one was measured).

In 2015 live trapping was used to minimize any effect the killing solution used in 2011 and desiccation caused by the tropical climate may have had on testes mass. Testes dissections were performed as quickly as possible after traps were collected and all beetles were processed within 72hrs of trap collection with any individuals remaining after this being released. There was no way to determine the length of time beetles had been within the trap before they were collected, therefore to minimize any bias caused by the effect of starvation on body and testes mass between species beetles from within replicates were processed in a random order. Prior to dissection beetles were kept in plastic containers which contained damp tissue paper before being euthanized by freezing and photographed as described above. The total mass of the individual was obtained and then testes were dissected out and weighed using a Sartorius BP2215 balance. A calibration weight was used before each measurement.

4.3.3 Molecular phylogeny

The vast majority of species investigated in this study have not had any previous molecular work performed on them and therefore no molecular phylogeny exists for the species studied here. We therefore constructed a phylogeny of tunneler species (see below for rationale) found within the study site using sequences of the 3' region of the mitochondrial cytochrome oxidase I (*coxI*) gene. Specimens used in making this phylogeny were trapped in 2015 and were the freshest available specimens that had been stored in 90% ethanol. Thirty-eight tunnelling species were available from 2015 collections and used to build the phylogeny. Three species of *Aphodius* were used as our outgroup, which were previously sequenced (Monaghan *et al.*, 2007) and the sequences downloaded from GenBank. *Aphodius* is presumed to be the sister taxon of the Scarabaeinae (Browne and Scholtz, 1999).

DNA was extracted using a Qiagen DNeasy blood and tissue kit following guideline protocols. Tissue was extracted from the flight and leg muscles unless individuals were small (pronotum width < 3mm): then the guts were removed and the remaining whole specimen was broken apart and used for DNA extraction. The *cox1* gene was amplified using primers Pat and Jerry (Simons *et al.*, 1994) and a Qiagen multiplex PCR kit, the guideline protocols were largely followed. In a few cases the annealing temperatures were adjusted and DNA template diluted to reduce contaminants. Reaction mix contained 12.5µl master mix, 5µl Q-solution, 2.5 – 3.125µl H₂O, 2.5µl primer mix (1.25µl Pat and 1.25µl Jerry) and 0.625 - 2.5µl DNA template. Cycling parameters were 15mins at 95°C followed by 30secs at 94°C, 90secs at 46-58°C and 90secs at 72°C repeated 35 times and 10mins at 72°C. A number of PCR products had low concentration and an additional PCR was performed on the template following the same protocol. Some PCR products had multiple bands after analysis by electrophoresis. By using a Monarch gel extraction kit the desired PCR product was isolated. All PCR products were purified using a Qiagen QIAquick PCR purification kit. Forward and reverse strands were sequenced using Sanger sequencing reaction. Chromatographs were assembled and edited in BioEdit and aligned using ClustalW (Thompson, Higgins and Gibson, 1994). Tree searches were performed on the web server T-REX (Alix, Boubacar and Vladimir, 2012) and tree selection using RAxML with the GTRCAT substitution model and hill climbing algorithm (Stamatakis, 2006) bootstrapped 1000 times.

4.3.4 Statistical analysis

4.3.4.1 Statistical rationale

Our study set out to test the persistence of species after the modification of habitat. Until relatively recently primary old growth forest would have dominated the majority of the landscape investigated in this study. We therefore only included species that were found in

OGF in our analysis, as it is likely species not found in OGF would have dispersed into these locations after habitat modification. Dung beetles can be separated into ecological guilds based on their method of dung removal and nesting behaviour (Slade *et al.*, 2007). Only paracoprid nesters (tunnelers) were used in our analysis. No telecoprid or endocoprid species (rollers and dwellers, respectively) possess horns, and their inclusion could potentially bias the results due to confounding effects caused by differences in burying depth of brood balls in soils with increased temperature in oil palm plantation (Edwards *et al.*, 2013) and increase the likelihood we would detect an effect of hornless species richness and abundances being reduced within OPP. Similar to previous findings (Edwards *et al.*, 2013; Gray *et al.*, 2014), in the current study only a single individual (*Sisyphus thoracicus*) was found in OPP which was not a tunneler. Species which were only represented by one individual across all trapping efforts in 2011 were also excluded from the analysis. This removed four *Onthophagus* species from the analysis. Three of these four *Onthophagus* species removed from analysis were also not trapped in 2015 and therefore material for the molecular phylogeny was not available for them.

Statistical analysis of diversity and abundance was performed using two approaches. The first approach, hereafter called the ecological dataset, incorporated the highest spatial resolution data at the trap level and fitted generalised linear mixed models, but did not take into account the phylogenetic relationships between species. The second approach, hereafter called the phylogenetic dataset, repeated the same analysis but took phylogenetic relationships between species into account by fitting phylogenetic generalised linear mixed models (Ives and Helmus, 2011). Due to increased model complexity it was only possible to fit these models by using the third highest spatial resolution at the replicate level. Additionally, we test for phylogenetic signal in morphological traits, assessing how similar traits within closely related species tend to be. In all cases, the phenotypic data used were the average trait values for

each species across all habitat types and years. We also determine the allometric relationship between horn length and body size for each species. All statistical analysis was carried out in R version 3.3.0 (R Development Core Team, 2016).

4.3.4.2 Horn length-body size allometry

If a species had low abundance and determining the allometric relationship between horn length and body size was difficult, we took additional photographs from specimens housed in the OUMNH to increase sample sizes. In all cases these extra individuals had been collected from within Sabah, Malaysian Borneo. The year of collection was not considered. Following visual inspection of log-log scatterplots there was clear evidence of non-linear scaling relationships between horn length and body size in the majority of species and recommendations outlined in Knell (2009a) to characterise non-linear allometries were carried out. Comparisons were made by fitting (1) simple linear models, (2) quadratic models, (3) breakpoint models fitted using the *Segmented* package in R (Muggeo, 2003) (4) sigmoidal models of the form:

$$\text{Horn length} = y_0 + \frac{a(\text{body size})^b}{c^b + (\text{body size})^b}$$

Where y_0 specifies minimum horn length, a the range of horn lengths, b specifies the slope coefficient and c the body size at the lower inflection of the curve (Moczek *et al.*, 2002) and (5) discontinuous models where morph (major or minor) was assigned by inspection of the frequency distribution of the ratio between horn length and body size (Cook & Bean, 2006) and added as an explanatory variable, including the interaction term with body size, and of the form:

$$\text{Horn length} = \text{body size} * \text{morph}$$

In a number of species not all models were necessary because, for example, allometry was clearly not sigmoidal based upon visual inspection. Fitted models were then compared on their goodness of fit using Akiaike's information criterion (AIC) and the model with the lowest AIC score selected, with a difference greater than 2 used to determine if a differences exists between them (Knell, 2009a). Species were then assigned a categorical variable which best described the scaling relationship between horn length and body size.

4.3.4.3 Ecological dataset

4.3.4.3.i Species richness

We analysed the ecological dataset using generalised linear mixed models (GLMM) with Poisson error structure using the *lme4* package (Bates *et al.*, 2015). We fitted a GLMM to the species richness for each trap with habitat and whether species were horned or hornless as categorical explanatory variables including their interaction term. Replicate was used as a random effect. Models fitted to the full dataset had convergence issues because there were no hornless species found in OPP. We therefore fitted a new model with OPP data removed. Comparison of both model summaries showed quantitatively similar results however we report the GLMM with OPP data removed.

4.3.4.3.ii Species abundance

Multiple separate GLMMs with Poisson error structure were fitted to abundance data to tests for associations between morphological traits and species abundance across habitat types. In all GLMMs replicate and species were used as random effects. Species was included as a random effect to account for variance caused by some species being abundant or rare. In all models an extra random effect of observation was added to account for overdispersion

(Harrison, 2014). GLMMs were fitted with habitat as a factor and the morphological trait in question, including their interaction term as explanatory variables. These GLMMs do not take into account phylogenetic relationships between species but have the greatest statistical power and highest level spatial resolution.

The morphological traits included as explanatory variables in GLMMs and in question whether there was an association with morphological trait and species abundance across habitat types were: (1) whether a species were horned or hornless; (2) the type of allometric relationship (continuous or discontinuous) between horn length and body size, species with discontinuous allometries tended to be larger therefore body size was included as fixed effect; (3) horn length (composite of total horn length) with male body size as a fixed effect; (4) sexual size dimorphism (SSD), measured as male body size minus female body size and (5) testes mass with male body mass as a fixed effect. All continuous explanatory variables were log transformed to improve model fitting.

4.3.4.4 Phylogenetic signal of traits

It has long been accepted that in comparative studies, individual species data points are not statistically independent due to the shared evolutionary histories of the species being studied. Taking into account a species phylogenetic relatedness is important as phenotypic trait divergence, if under Brownian motion, is proportional to how closely related species are (Harvey and Pagel, 1991). We assessed the phylogenetic signal of each phenotypic trait, put simply how similar are the traits of closely related species. We assess phylogenetic signal using the *phylosig* function in the *phylotools* package (Revell, 2012) to estimate Pagel's λ (Pagel, 1999) and Blomberg's K (Blomberg, Garland and Ives, 2003). λ assesses the phylogenetic correlation between species and scales between 0 and 1 representing no correlation between species and Brownian motion, respectively (Pagel, 1999). K is a scaled

ratio of trait variation between species compared to a null model of contrast variance expected under Brownian motion, with values of 1 indicating correlation between species equal to a Brownian motion model and values less or greater than 1 indicating less or more phylogenetic signal, respectively (Blomberg, Garland and Ives, 2003). We then test using maximum likelihood whether λ is significantly different to when values of λ are forced to 0 and 1 and test whether K is significantly different from 0.

4.3.4.5 Phylogenetic dataset

We then repeated the analysis of species abundance (above) but this time incorporated phylogenetic relationship of species into our models. As explained above, models could not be fitted to the full dataset and so data from each replicate was amalgamated. Using the *pez* package in R (Pearse *et al.*, 2015) phylogenetic generalised liner mixed models (PGLMM) were fitted which allow regression of species abundance against trait values whilst using a matrix of species relatedness derived from phylogenetic data as a random effect (Ives and Helmus, 2011). Models were fitted with the same explanatory variables as in the ecological dataset except that continuous variables were standardised to have a mean of zero. PGLMM cannot be fitted using Poisson errors therefore we log transformed abundance plus one. Models including and excluding interaction terms between phenotypic trait and habitat explanatory variables were compared using AIC scores, with the model with lowest AIC score selected as the best fitting model.

4.4 Results

4.4.1 Species summary

We restricted our analysis to 30 species (Table 4.1). Nine species were hornless and 21 were horned. In three of these 21 horned species females also express horns, in all of these cases the largest females' horns were approximately the same size as the smallest males' horns and of similar structure (personal observation) and are not considered further here. It was possible to determine the type of allometric relationships between horn length and body size in 16 species, 9 of which showed discontinuous and 7 of which showed sigmoidal allometries (Figure 4.2). In total the horn length of 20 species, testes mass of 26 species and SSD of 29 species were determined and used in subsequent results (Table 4.1).

4.4.2 Molecular phylogeny

A total of 766 base pairs from the *cox1* gene of 36 tunneler species were sequenced and compared to 3 species of *Aphodius* used as an outgroup, only the phylogram of the 30 species included in our dataset is presented (Figure 4.3; see Appendix Figure 4.3A for full phylogram). The most parsimonious tree is presented, however base nodes and a number of external nodes had weak bootstrap support (<50% bootstrap values) and these nodes have been collapsed. Therefore determining the relationship between genera is difficult; however genetic distances can still be used. Except in one case (*O. laevis*) species of the same genus were grouped together. The nodes remaining on the phylogram (>50% bootstrap values) generally showed strong support all having >85% bootstrap values.

Table 4.1: Summary data of 30 dung beetle species used in analysis.

Species	Total trapped individuals (2011)	Horned or hornless	Horn type	Allometry	Female body size mm (n)	Male body size mm (n)	Horn length mm (n)	Testes mass mg (n)	Body mass mg (n)	Habitats present
<i>Caccobius bawangensis</i>	7	horned	H-1	-	1.748 (3)	1.677 (13*)	0.27 (13*)	-	-	OGF, SLF, HLF, OPP
<i>Catharsius dayacus</i>	346	horned	H-1 P-2	discontinuous	15.366 (113)	15.193 (217)	7.32 (217)	20.42 (80)	1898.843 (80)	OGF, HLF, SLF
<i>Copris agnus</i>	33	horned	H-1	discontinuous	8.362 (17)	8.546 (33)	0.887 (33)	3.3 (14)	347.271 (14)	OGF, SLF, HLF
<i>Copris sinicus</i>	181	horned	H-1 P-s	discontinuous	8.667 (75)	9.244 (77)	1.892 (77)	2.71 (10)	365.57 (10)	OGF, SLF, HLF
<i>Microcopris doriae</i>	251	horned	H-1	discontinuous	6.773 (82)	7.009 (146)	0.758 (146)	2.455 (33)	156.29 (33)	OGF, SLF, HLF
<i>Microcopris hidaki</i>	93	hornless	-	-	4.404 (69)	4.315 (71)	-	0.888 (26)	38.45 (26)	OGF, SLF, HLF
<i>Onthophagus angustatus</i>	8	horned	H-2	-	4.967 (5)	5.329 (2)	4.048 (2)	-	-	OGF, HLF
<i>Onthophagus aphodiodes</i>	52	hornless	-	-	2.511 (21)	2.581 (30)	-	0.5 (3)	6.333 (3)	OGF, SLF, HLF
<i>Onthophagus borneensis</i>	33	horned	H-2	discontinuous	5.558 (19)	5.291 (68*)	1.558 (68*)	2.505 (20)	74.485 (20)	OGF, SLF, HLF
<i>Onthophagus cervicapra</i>	633	horned	H-2 P-s	continuous	3.718 (301)	3.744 (405)	1.256 (405)	1.02 (143)	28.434 (143)	OGF, SLF, HLF, OPP
<i>Onthophagus clivemurus</i>	7	horned	H-1	-	3.374 (6)	-	-	-	-	OGF
<i>Onthophagus fujii</i>	42	horned	H-2 P-s	continuous	3.777 (19)	3.781 (94*)	1.126 (94*)	1.039 (33)	29.212 (33)	OGF, SLF, HLF, OPP
<i>Onthophagus incicus</i>	62	horned	H-2 P-2	discontinuous	6.705 (63)	6.689 (42*)	3.799 (42*)	2.967 (6)	168.067 (6)	OGF, SLF, HLF, OPP
<i>Onthophagus kawahari</i>	22	hornless	-	-	2.227 (14)	2.369 (7)	-	-	-	OGF, SLF
<i>Onthophagus laevis</i>	2	horned	H-1	-	4.615 (7)	4.664 (12)	0.329 (12)	1.227 (11)	54.855 (11)	OGF, HLF
<i>Onthophagus negroboscior</i>	65	horned	H-2	continuous	3.534 (82)	3.643 (113)	1.401 (113)	0.942 (79)	24.45 (79)	OGF, SLF
<i>Onthophagus nr.borneensis</i>	19	hornless	-	-	5.748 (23)	5.649 (29)	-	2.826 (20)	83.875 (20)	OGF, SLF, HLF
<i>Onthophagus obscurior</i>	1408	horned	H-2	continuous	3.57 (474)	3.609 (835)	1.456 (835)	0.951 (163)	26.163 (163)	OGF, SLF, HLF, OPP
<i>Onthophagus ochromerus</i>	53	horned	H-1	discontinuous	4.025 (23)	4.095 (48*)	0.76 (48*)	1.638 (13)	40.131 (13)	OGF, SLF, HLF
<i>Onthophagus pacificus</i>	55	hornless	-	-	3.454 (37)	3.388 (91)	-	0.621 (61)	18.402 (61)	OGF, SLF, HLF
<i>Onthophagus pavidus</i>	17	horned	H-2	-	4.236 (9)	3.984 (12*)	2.176 (12*)	1.2 (2)	35.45 (2)	OGF, SLF, HLF, OPP
<i>Onthophagus rorarius</i>	88	horned	H-2	discontinuous	6.03 (67)	5.772 (41)	2.849 (41)	2.024 (17)	110.824 (17)	OGF, SLF, HLF, OPP
<i>Onthophagus rudis</i>	30	hornless	-	-	3.158 (14)	3.091 (64)	-	0.579 (47)	20.698 (47)	OGF, SLF, HLF
<i>Onthophagus rugicollis</i>	734	horned ^f	H-2	continuous	4.524 (276)	4.511 (467)	3.315 (467)	1.328 (111)	52.686 (111)	OGF, SLF, HLF, OPP
<i>Onthophagus sarawacus</i>	11	hornless	-	-	5.899 (11)	6.08 (3)	-	2.5 (3)	107.333 (3)	OGF, SLF
<i>Onthophagus semiaureus</i>	12	hornless	-	-	5.551 (7)	5.487 (17)	-	2.590 (11)	108.990 (11)	OGF, SLF, HLF
<i>Onthophagus semicupreus</i>	16	hornless	-	-	3.507 (8)	3.677 (12)	-	1.4 (1)	52.3 (1)	OGF, HLF
<i>Onthophagus vulpes</i>	436	horned ^f	H-2	continuous	4.225 (136)	4.167 (326)	2.374 (326)	1.347 (96)	45.622 (96)	OGF, SLF, HLF, OPP
<i>Onthophagus waterstradi</i>	33	horned	H-1	continuous	3.431 (22)	3.562 (58*)	0.474 (58*)	0.897 (30)	25.317 (30)	OGF, SLF, HLF, OPP
<i>Proagoderus watanabei</i>	444	horned ^f	H-2 P-1	discontinuous	9.734 (277)	9.658 (304)	13.469 (304)	4.891 (121)	368.534 (121)	OGF, SLF, HLF, OPP

All values are average values from pooled data. Horn length is composite length of all horns a species possess.

Horn type: H = head horns, P = pronotum horns and number of horns: 1 = single, 2 = paired, s = sculpturing.

(n*) = supplemented with specimens from OUMNH and horned^f = females also possess horns.

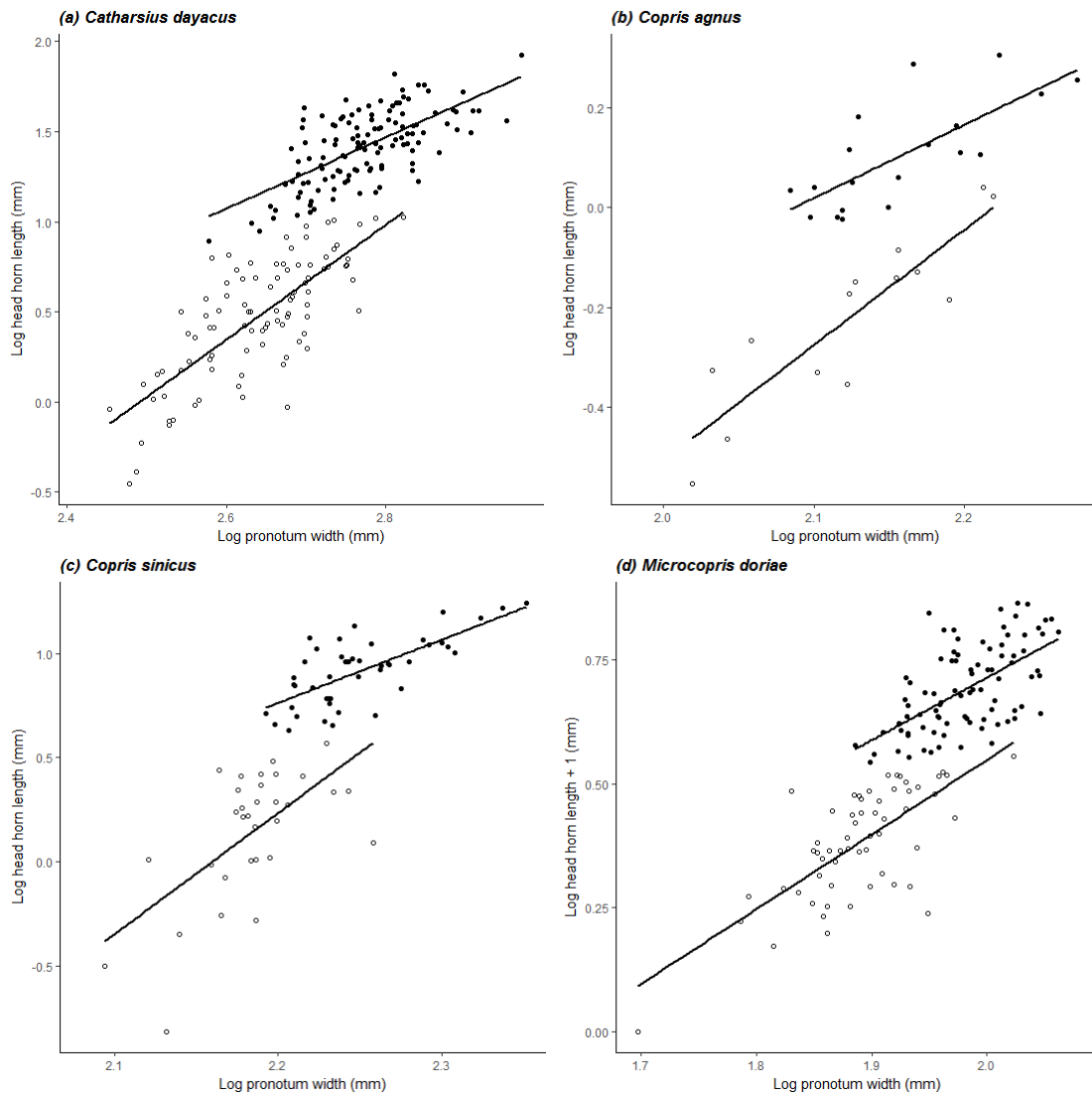


Figure 4.2: log-log scatterplots of the 16 species that it was possible to determine the allometric relationship between head horn length and body size. Species with discontinuous allometries (a-i), minors and majors are indicated by hollow and solid circles, respectively. Species with continuous sigmoidal allometries (j-p) the dashed line indicates the switchpoint body size at which males larger than this body size are classified as majors. The switchpoint was estimated using *Segmented* (Muggeo, 2003) after removal of asymptotic majors males as suggested by Knell (2009a).

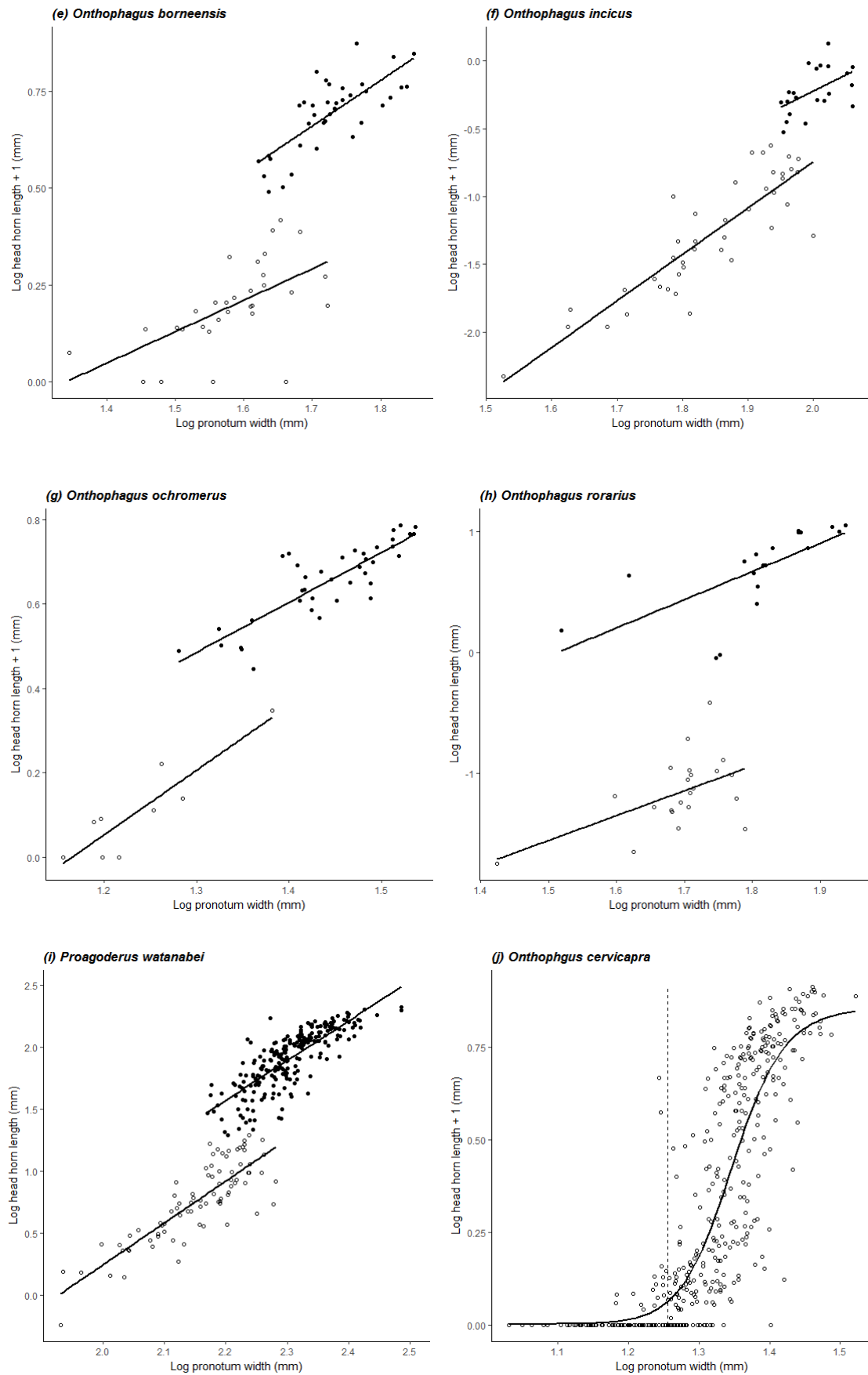


Figure 4.2. (continued).

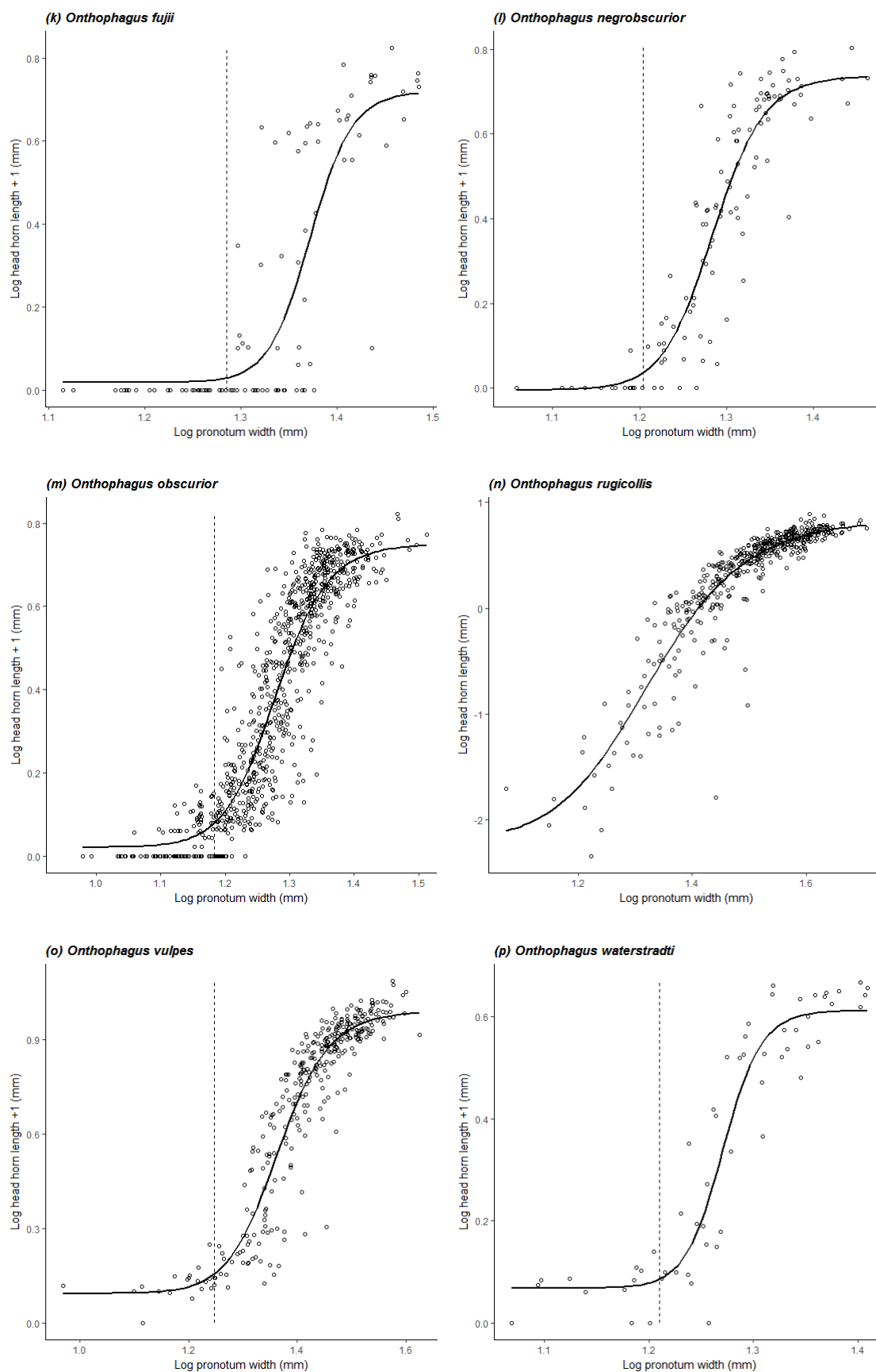


Figure 4.2. continued.

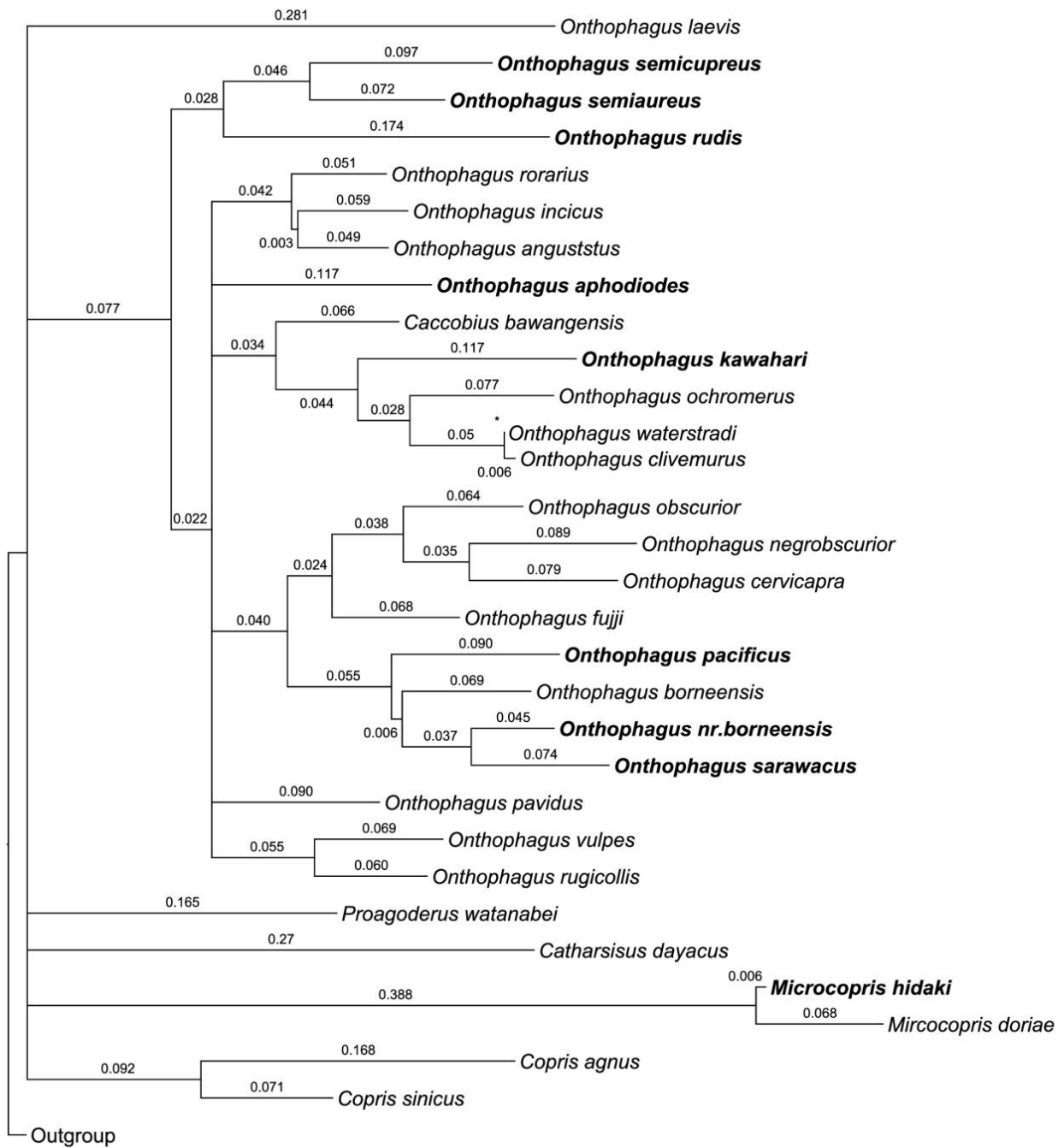


Figure 4.3: Phylogram made using TreeGraph 2 (Stöver and Müller, 2010) of maximum likelihood tree of the *cox1* gene the 30 dung beetle species used in analysis and rooted using sequences of three *Aphodius* species as an outgroup. Hornless species are in bold. Nodes with <50% bootstrap values have been collapsed. Numbers indicate the genetic distance for each branch, the branch labelled with an asterisk has a value of 6.2×10^{-6} .

4.4.3 Phylogenetic signal

Assessing the phylogenetic signal using Pagel's λ and Blomberg's K showed quantitatively similar results (Table 4.2). Overall, relative horn length and relative testes mass showed strong phylogenetic signal, allometry and presence/absence of horns showed weak phylogenetic signal and SSD showed very little phylogenetic signal.

Table 4.2: Evaluation of the phylogenetic signal of each morphological trait.

Trait	Pagel's λ			Blomberg's K	
	λ	$\ln L \lambda = 0$ (p value)	$\ln L \lambda = 1$ (p value)	K	p value
Horns	0.73	-18.56 (0.213)	-19.01 (0.116)	0.59	0.09
Allometry	0.72	-11.61 (0.113)	-10.73 (1)	1.01	0.013
Horn length	0.99	-6.52 (0.007)	-2.82 (1)	0.90	0.077
Testes mass	0.99	72.64 (0.005)	76.57 (1)	1.05	0.011
SSD	0.22	5.52 (0.778)	2.18 (0.009)	0.42	0.719

λ - p values show λ is significant differences when using likelihood ratio tests compared to models when $\lambda = 0$ (no phylogenetic signal) and when $\lambda = 1$ (strong phylogenetic signal).

K - p values show when K is significant difference from $K = 0$

4.4.4 Ecological dataset

4.4.4.1 Species richness

The minimal adequate model fitted to the species richness data retained the two-way interaction between horn presence/absence and habitat ($\chi^2 = 9.64$, $df = 1$, $p = 0.008$; Figure 4.4). Inspection of the model summary indicates that overall species richness of hornless species is lower than horned species. Further inspection of model summary and figure 4.4 shows that selectively logged forest (SLF) retains a similar species richness of horned species

as old growth forest (OGF) but significantly fewer hornless species persist. When forest is heavily logged (HLF) there was an overall reduction in both horned and hornless species persisting from OGF indicated by the model summary. Although our analysis on the restricted dataset excluded the oil palm plantation (OPP) data, it is clear from figure 4.4 that overall species richness is further reduced in OPP compared to all other habitat types. Strikingly, no hornless species found in OGF were also found in OPP. In contrast, on average approximately 4 horned species are found in each OPP trap and over half (11 of 21) of all horned species found in OGF persist within OPP (Table 4.1).

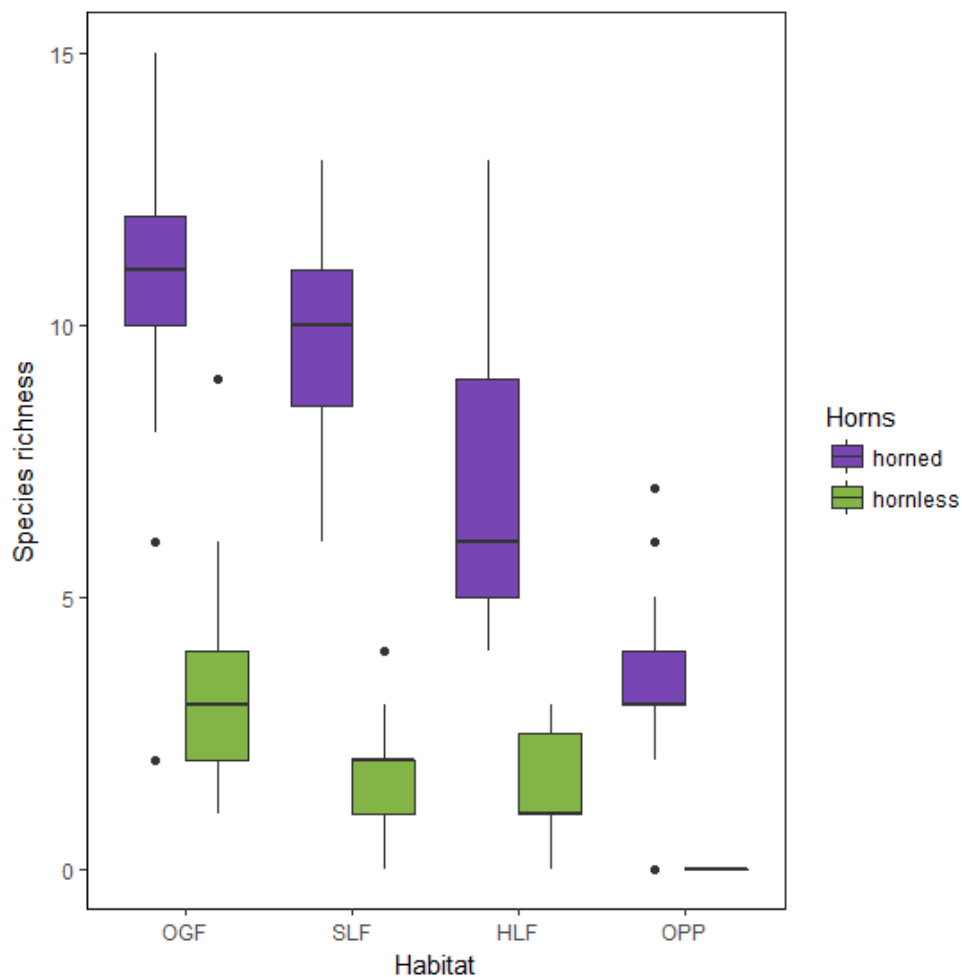


Figure 4.4: Species richness of horned (purple) and hornless (green) species per trap in old growth forest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP).

4.4.4.2 *Species abundance*

The fitted model assessing species abundance retained the two-way interaction between horn presence/absence and habitat ($\chi^2 = 105.63$, $df = 1$, $p < 0.001$; Figure 4.5). The model summary indicates that the abundances of horned and hornless species in OGF are not significantly different and the two-way interaction is caused by a reduction in abundance of hornless species in SLF and HLF while horned species maintain similar abundances to that found in OGF. The abundance of both horned and hornless species is reduced in OPP but this reduction in abundance is significantly less in horned species.

Of those horned species for which a horn length-body size allometry type was able to be determined, species with discontinuous allometries were significantly larger than those with continuous allometries ($F = 2405.7$, $df = 1$, $p < 0.001$). There was an effect of allometry type on the abundance of species across the disturbance gradient indicated by the minimal adequate model retaining the two-way interaction between allometry type (continuous or discontinuous) and habitat ($\chi^2 = 145.11$, $df = 1$, $p < 0.001$; Figure 4.6). Body size was used as a fixed effect and was close to significant ($\chi^2 = 3.72$, $df = 1$, $p = 0.054$). Species with continuous allometries were more abundant than species with discontinuous allometries in OGF, whereas in SLF there was only a reduction in abundance of species with continuous allometries. The two-way interaction however, appears to be mainly driven by species with discontinuous allometries becoming less abundant in the most disturbed habitats (HLF and OPP) when compared to species with continuous allometries which maintained similar abundances in HLF as found in OGF. Although species with continuous allometries also became less abundant in OPP, the reduction in abundance is significantly less when compared to those species with discontinuous allometries.

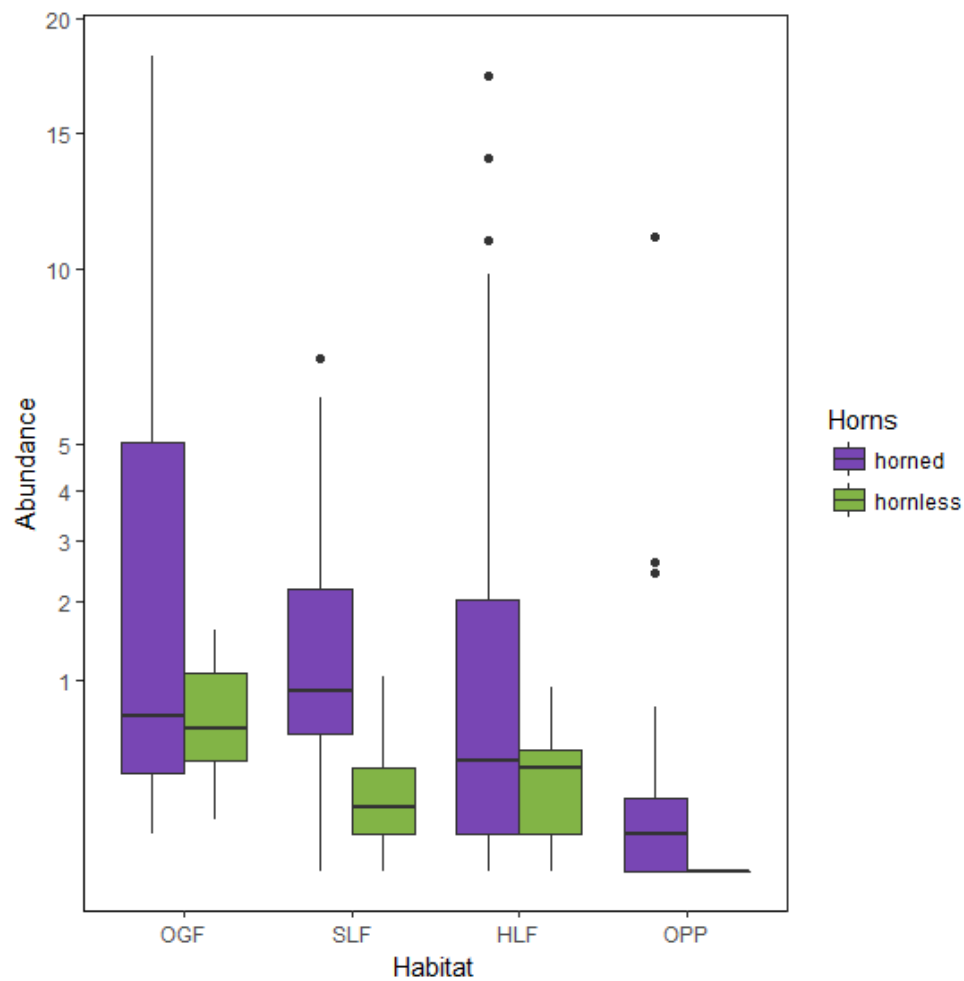


Figure 4.5: Abundance of horned (purple) and hornless (green) species per trap in old growth forest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP). Note that the y-axis is on a square root scale but abundance data are untransformed.

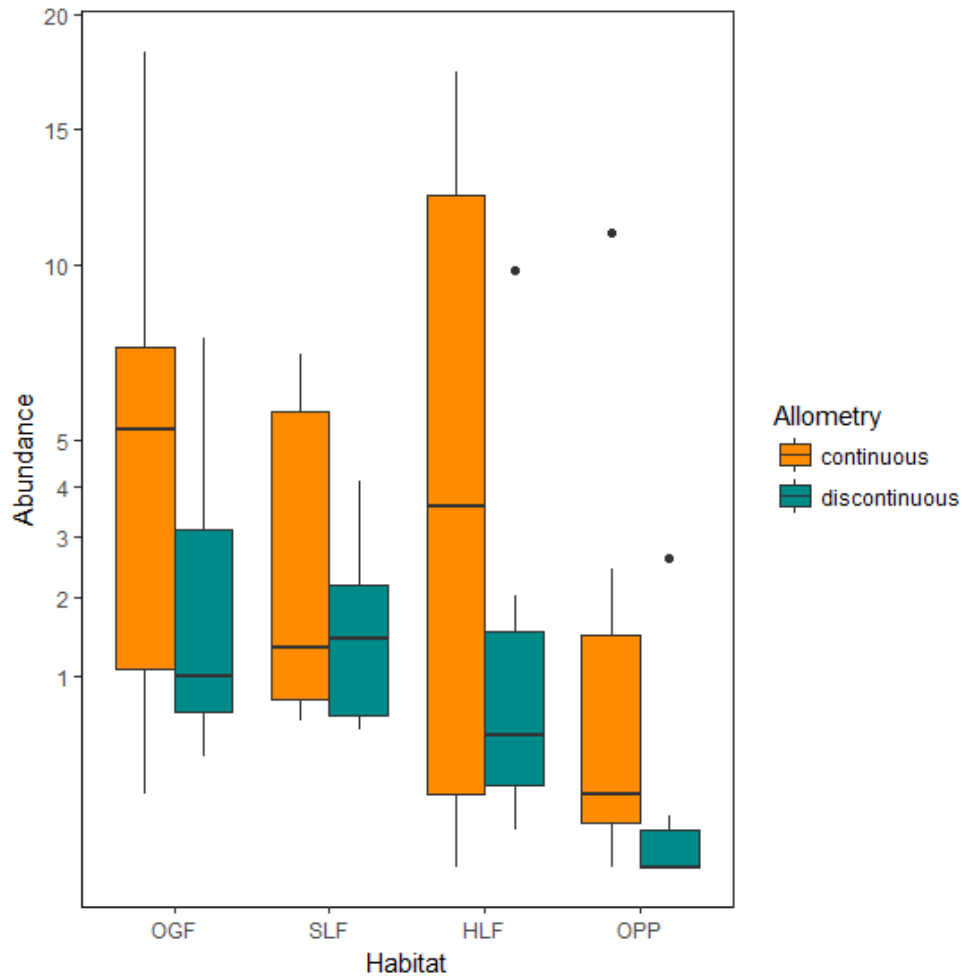


Figure 4.6: Abundance of horned species with continuous (orange) and discontinuous (green) allometries per trap in old growth forest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP). Note that the y-axis is on a square root scale but abundance data are untransformed.

Within horned species, a significant two-way interaction between relative horn length and habitat was retained in the minimum adequate model and indicates that horn length is associated with species abundance across some habitats ($\chi^2 = 21.60$, $df = 1$, $p < 0.001$; Figure 4.7). Inspection of figure 4.7 and model summary indicates there was no association between relative horn length and species abundance in OGF and the two-way interaction is driven by an association of relative horn length and abundance in altered habitats (SLF, HLF and OPP).

Those species with relatively short horns tend to have reduced abundances in SLF, HLF and most obviously in OPP. In contrast, those species with relatively large horns tend to persist and maintain similar abundances to what was observed in OGF within these altered habitats.

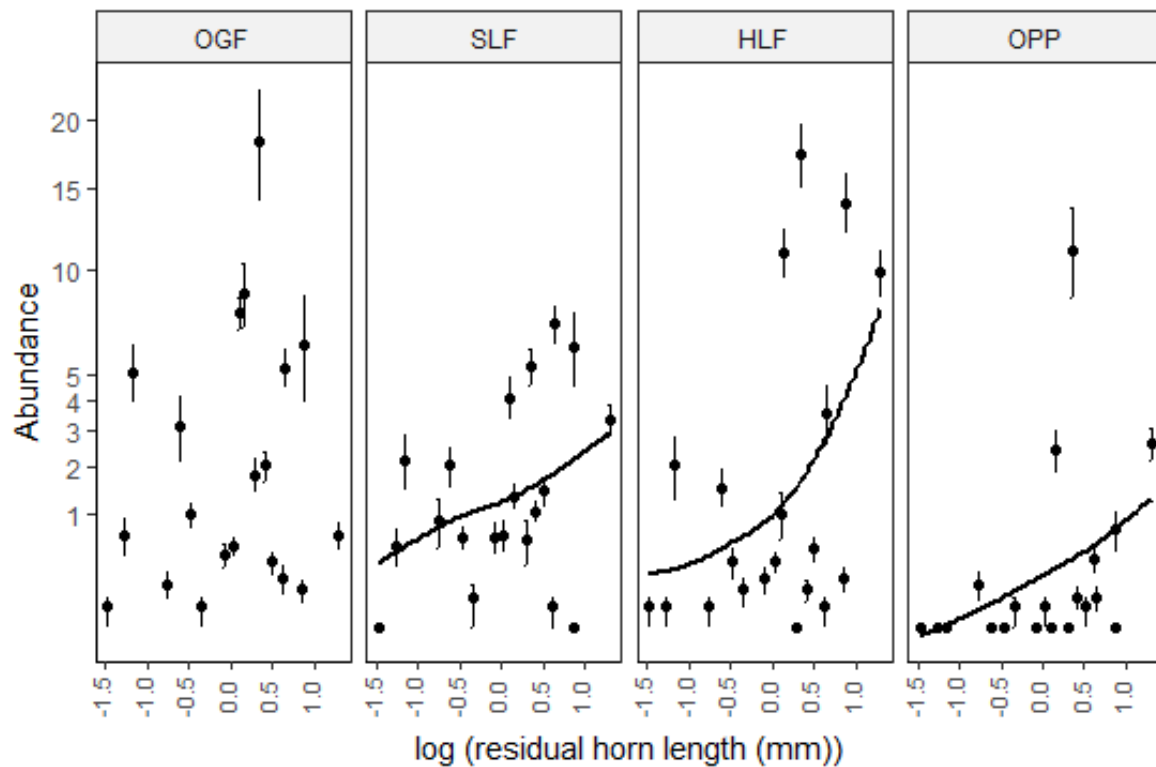


Figure 4.7: The effect of horn length controlling for body size on species abundance across the disturbance gradient. Beetles were sampled from old growth forest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP). Lines are fitted using a loess smoother and only those lines significantly different from zero based on GLMM analysis are displayed. Note that the y-axis is on a square root scale but abundance data are untransformed.

Sexual size dimorphism (SSD) was found to have no effect on the abundance of species in any habitat type. The interaction between habitat and SSD was close to significance ($\chi^2 = 6.93$, $df = 1$, $p = 0.074$; Figure 4.8), and inspection of the model summary suggests that species with larger males relative to females suffer decreased abundance in OPP but given the high p-value we can have little confidence in this particular pattern. The main effect of SSD was also non-significant ($\chi^2 = 0.01$, $df = 1$, $p = 0.904$).

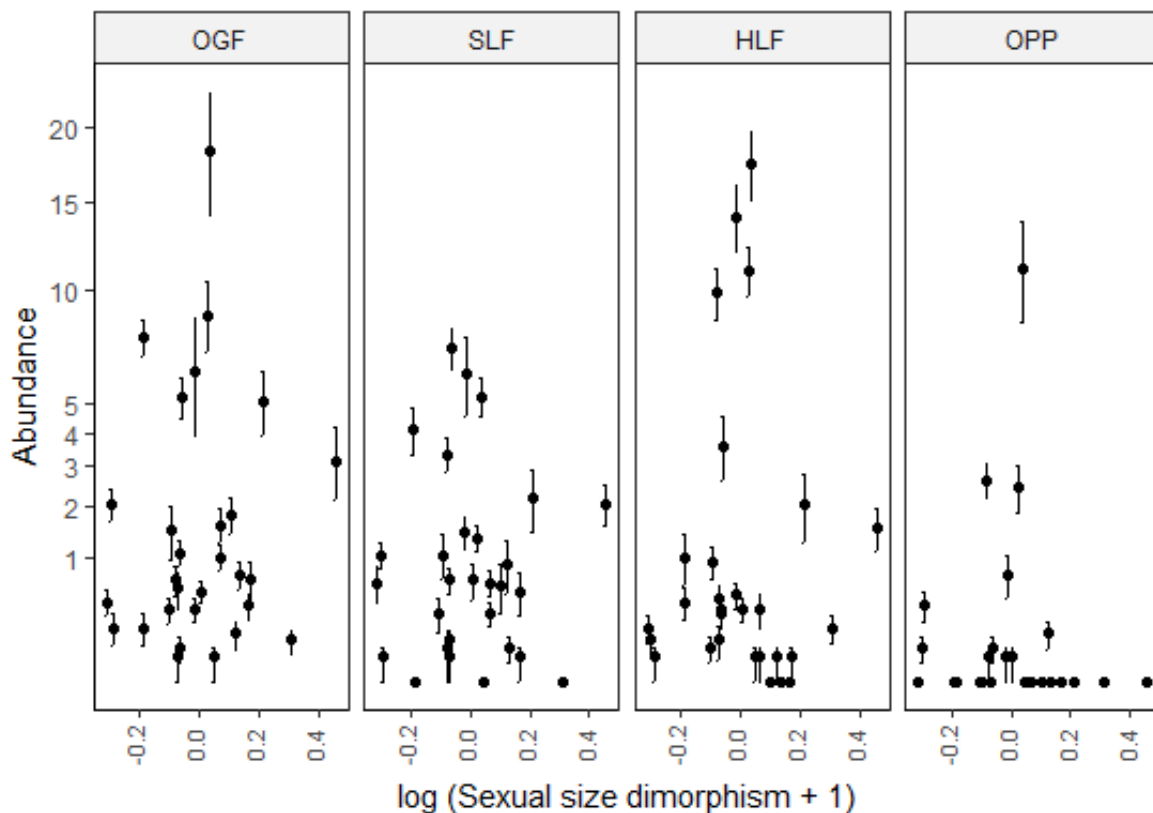


Figure 4.8: The effect of sexual size dimorphism (SSD) on species abundance across the disturbance gradient. Positive values indicate species with larger average male body size and negative values species with larger average female body size. Beetles were sampled from old growth forest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP). No trend lines are fitted due to none being significantly different from zero based on GLMM analysis. Note that the y-axis is on a square root scale but abundance data are untransformed.

A significant two-way interaction was found in the model containing testes mass as an explanatory variable and habitat on species abundance ($\chi^2 = 18.89$, $df = 1$, $p < 0.001$; Figure 4.9), after controlling for body mass. Inspection of model estimates show that the intercepts are reduced in SLF, HLF and OPP compared to OGF. The slope coefficient within OGF was weakly negative, the slope coefficient became increasingly negative in HLF and OPP and a slight positive increase in SLF. However, reordering the model summary table shows that none of the line coefficients from each habitat are significantly different from zero. Further inspection of figure 4.9 indicates that it is those species with the most average testes mass which have the highest abundance. This is most obvious in OPP; because of this it seems likely that finding the significant two-way interaction is probably caused by reduction in abundances of those species with the most positive and negative residual testes mass having a large effect on the model. Analysis on the effect of testes mass when controlling for body mass on species abundance within each habitat separately and across all habitats (i.e. interaction term removed from model) indicates that within each habitat there is no effect of testes mass on the abundance of species (all habitats: $p = 0.91$; OGF: $p = 0.99$; SLF: $p = 0.93$; HLF: $p = 0.48$; OPP: $p = 0.95$).

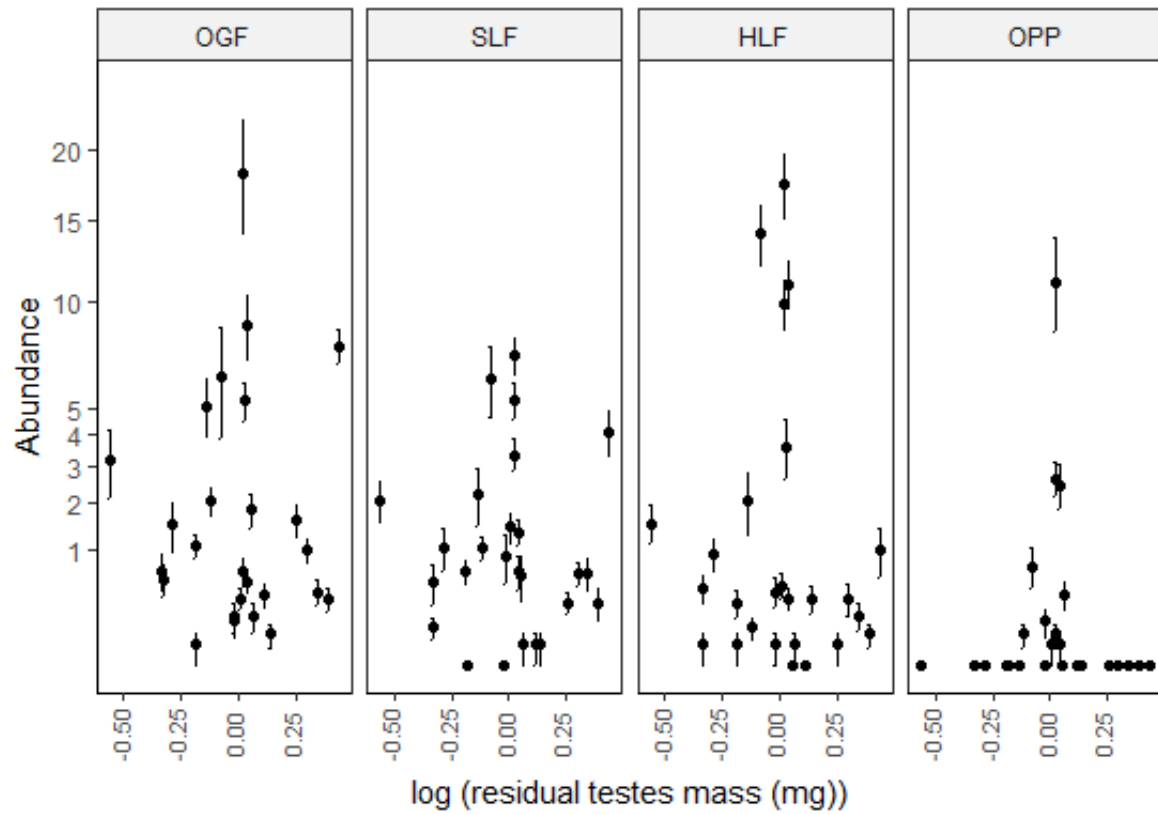


Figure 4.9: The effect of testes mass controlling for body mass on species abundance across the disturbance gradient in old growth forest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP). No trend lines are fitted due to none being significantly different from zero based on GLMM analysis. Note that the y-axis is on a square root scale but abundance data are untransformed.

4.4.5 Phylogenetic dataset

Fitting PGLMMs with the phylogenetic dataset which control for phylogenetic relatedness between species, show similar results to the ecological dataset (Table 4.3). When considering the abundance of horned and hornless species across the disturbance gradient, the preferred PGLMM on the basis of AIC score was the model retaining the interaction term between horn presence/absence and habitat type. The model coefficients indicate that the abundance of hornless species is reduced as habitat disturbance increases and that the two-way interaction is largely driven by horned species in SLF maintaining approximately similar abundance compared to what was seen in OGF. In HLF and OPP the coefficients from the fitted PGLMM indicate that horned species abundance is also reduced. By contrast, when the GLMM was fitted to the ecological dataset model coefficients indicated that in HLF horned species maintained similar abundances to what was found in OGF, whereas hornless species abundance was reduced.

Moving to the analysis of horn length effects within the horned species, the PGLMM with the interaction term between horn length when controlling for body size and habitat had a lower AIC score than the model fitted without the interaction term. The model summary indicates very similar trends to the GLMM fitted with the ecological dataset with species possessing relatively large horns persisting at higher abundance in disturbed habitats than those with relatively small horns. This difference is significant in SLF and only marginally non-significant in HLF despite having a larger effect size. There was however no longer an effect of relative horn length within OPP as seen in the ecological dataset.

Table 4.3: Summary of PGLMMs. AIC scores with and without the interaction term between phenotypic trait and habitat type are shown for comparison. Summary table of SSD displayed has non-significant interaction term removed but shows non-significant main effect of SSD.

Horns						
	Model summary with interaction term				AIC scores	
	Estimate	SE	Z score	<i>p</i> value	with	without
Intercept	1.438	0.488	2.944	0.003	965.9	969.4
Habitat - SLF	-0.634	0.340	-1.865	0.062		
Habitat - HLF	-0.632	0.460	-1.372	0.170		
Habitat - OPP	-1.293	0.611	-2.116	0.034		
Horns - horned	0.624	0.508	1.228	0.220		
Habitat - SLF * Horns- horned	0.493	0.285	1.731	0.083		
Habitat - HLF * Horns- horned	0.252	0.387	0.659	0.515		
Habitat - OPP * Horns- horned	0.168	0.515	0.236	0.744		
Allometry						
	Model summary with interaction term				AIC scores	
	Estimate	SE	Z score	<i>p</i> value	with	without
Intercept	3.403	0.644	5.256	<0.001	544.2	550.2
Habitat - SLF	-0.412	0.438	-0.941	0.347		
Habitat - HLF	-0.423	0.584	-0.724	0.469		
Habitat - OPP	-1.476	0.768	-1.921	0.055		
Allometry - discontinuous	-1.194	0.818	-1.459	0.145		
log (body size)	0.428	0.360	1.190	0.234		
Habitat - SLF * Allometry- discontinuous	0.407	0.394	1.032	0.302		
Habitat - HLF * Allometry- discontinuous	-0.480	0.544	-0.883	0.377		
Habitat - OPP * Allometry- discontinuous	-0.544	0.728	-0.748	0.454		
Horn length						
	Model summary with interaction term				AIC scores	
	Estimate	SE	Z score	<i>p</i> value	with	without
Intercept	2.416	0.503	4.804	<0.001	662	672.7
Habitat - SLF	-0.244	0.350	-0.700	0.485		
Habitat - HLF	-0.639	0.484	-1.320	0.187		
Habitat - OPP	-1.565	0.649	-2.413	0.016		
log (horn length)	0.188	0.402	0.466	0.641		
log (body size)	-0.106	0.360	-0.293	0.769		
Habitat - SLF * log (horn length)	0.358	0.174	2.054	0.040		
Habitat - HLF * log (horn length)	0.481	0.254	1.897	0.058		
Habitat - OPP * log (horn length)	0.198	0.347	0.571	0.568		

Table 4.3: continued.

Sexual size dimorphism (SSD)						
	Model summary without interaction term				AIC scores	
	Estimate	SE	Z score	<i>p</i> value	with	without
Intercept	1.950	0.336	5.800	<0.001	946.6	946.8
Habitat - SLF	-0.295	0.273	-1.080	0.280		
Habitat - HLF	-0.496	0.359	-1.382	0.167		
Habitat - OPP	-1.271	0.468	-2.714	0.007		
log (sexual size dimorphism)	-0.007	0.214	-0.034	0.973		

Testes size						
	Model summary with interaction term				AIC scores	
	Estimate	SE	Z score	<i>p</i> value	with	without
Intercept	2.027	0.358	5.656	<0.001	854	860.3
Habitat - SLF	-0.228	0.287	-0.793	0.428		
Habitat - HLF	-0.437	0.383	-1.143	0.253		
Habitat - OPP	-1.235	0.503	-2.455	0.014		
log (testes mass)	-0.755	1.475	-0.512	0.609		
log (body mass)	1.071	1.470	0.729	0.466		
Habitat - SLF * log (testes mass)	0.067	0.139	0.479	0.632		
Habitat - HLF * log (testes mass)	-0.253	0.180	-1.409	0.159		
Habitat - OPP * log (testes mass)	-0.383	0.232	-1.649	0.099		

When the type of allometric relationship between horn length and body size was included as an explanatory variable, the model with the interaction term between type of allometry and habitat has a better fit than the PGLMM without the interaction. The model summary indicates that species with discontinuous allometries have reduced abundance in the most disturbed sites (i.e. HLF and OPP). By contrast with the results from the non-phylogenetically controlled analysis, however, the difference in abundance between species with continuous and discontinuous allometries was no longer significant in OGF and SLF.

The PGLMMs with and without the interaction term between SSD and habitat type were indistinguishable from each other based on AIC scores, showing the same lack of relationship between SSD and abundance across habitat types as when the GLMM was fitted.

Additionally, the fitted PGLMM retaining the interaction between testes mass when controlled with body mass and habitat type had a lower AIC score compared to the PGLMM

without the interaction term. The model summary indicates that that only a weak effect exists and that species with relatively large testes have a reduced abundance within OPP. The effects are in the same direction as in the GLMM and similarly with the ecological dataset, when the model summary was reordered so that SLF, HLF and OPP were the intercepts all slope coefficients did significantly differ from zero, further indicating that there is only a very weak effect of testes of species abundance.

4.5 Discussion

The rapid increase in logging activities and conversion of tropical forest to agriculture is clearly a concern for global biodiversity. In line with previous research (Gray *et al.*, 2014; Slade *et al.*, 2011) and as predicted, we find that as logging intensity increases and forest is converted to agriculture there is a general reduction in the persistence of those dung beetle species which are found in old growth forest across the disturbance gradient. This reduction in species richness and abundance was however, generally less in species which express horns, compared to hornless species. Furthermore, we find that those horned species with relatively large horns tend to maintain similar abundances in disturbed habitats to that seen in old growth forest, and that on average it is species with relatively small horns that have the greatest reduction in abundance moving along the gradient of habitat modification.

In line with predictions, these results suggest that the intensity of pre-copulatory sexual selection a species experiences can enhance the persistence and possibly adaptive capacity of populations when facing environmental change, likely due to increased skew in male reproductive success. This is consistent with theory (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016) and laboratory studies (Fricke and Arnqvist, 2007; Plesnar-Bielak *et al.*, 2012; Jacomb, Marsh and Holman, 2016). However, these data contradict previous field studies which tend to find that the expression of sexually selected traits is negatively related to persistence (e.g. Sorci, Møller and Clobert, 1998) and are in direct contrast to the finding that horn length in bovids is positively associated with extinction risk (Bro-Jørgensen, 2014).

Contrary to our predictions the lack of a strong effect of SSD and testes mass on species persistence indicates that not all aspects of sexual selection influence adaptation or extinction risk. The possibility that the costs and benefits associated with different sexually selected traits may differ and be trait specific are in line with a number of previous comparative

studies. For example, horn length but not SSD in bovids was found to be associated with extinction risk (Bro-Jørgensen, 2014) and similarly in birds SSD and sexual dichromatism were not associated with extinction risk but testes size was (Morrow and Pitcher, 2003).

The evolution and exaggeration of horns is thought to be linked with species ecology and the ability of males to monopolise females (Emlen & Oring, 1977; Kokko & Rankin, 2006) and it has been argued that the reproductive benefits of aggressive male behaviour and weaponry will generally only outweigh their costs and be selected for at intermediate population densities (Knell, 2009b). Increasing group size in bovids is positively correlated with horn length, indicating that investment in horns is favoured with increasing competition (Bro-Jørgensen, 2007). By contrast, in a South African dung beetle community species with high male crowding and population density were more likely to be hornless, possibly due to males being unable to monopolise females and traits associated with scramble and sperm competition being favoured (Pomfret and Knell, 2008). Our data from undisturbed habitat (i.e. OGF), which is assumed to be a baseline of species abundance, do not however support an association between population density and the expression and exaggeration of horns, suggesting other aspects of species ecology may be influencing whether or not males are able to monopolise females. Species may differ in dung preferences (Whipple and Hoback, 2012) which could affect the heterogeneity of resources or species may navigate differently within the spatially complex environment of tropical forest, both of which could alter the fitness consequences of aggressive behaviour and weaponry (Forsgren, Kvarnemo and Lindstrom, 1996; Tomkins *et al.*, 2011).

Our data provides evidence that species in which males engage in contest competition, predicted from the presence of horns, and in which the intensity of contest competition is increased, predicted by relative horn lengths, generally have an enhanced ability to persist during environmental change. Based on our predictions this indicates that the costs of

expressing horns in altered habitats are largely outweighed by the benefits of increased sexual selection on population fitness and their persistence within altered habitats. In dung beetles male competitiveness is linked to the strength of males in pushing contests within tunnels (Moczek and Emlen, 2000). Body size has been reported to be an important factor in contest competition and there is evidence that in at least the largest males horn length is an important predictor of contest outcomes (Moczek and Emlen, 2000; Pomfret and Knell, 2006). It has been shown that strength is condition dependant (Almbro and Simmons, 2014) especially in males expected to be engaging in fighting (Knell and Simmons, 2010) and horn length is largely based upon larval resources, suggesting condition dependence (Emlen, 1996). It seems likely that reproductive skew will be increased in horned compared to hornless species and further increased in those species with larger horns because males are more likely to be able to exclude rivals from gaining copulations. Due to the condition dependency of pre-copulatory sexually selected traits, this reproductive skew is likely to favour the largest males with the largest horns (Hunt and Simmons, 2001), thus increasing the spread of beneficial adaptive alleles throughout the population, driving local adaptation and potentially increasing niche breadth (Proulx, 1999, 2002; Lorch *et al.*, 2003).

In all horned species with a large enough sample size for horn length-body size allometry to be determined, a non-linear scaling relationship between horn length and body size was found, evidence that the majority show polyphenism in male populations, a phenomenon widely reported in dung beetles and other insect taxa (Cook & Bean, 2006; Emlen, Hunt, *et al.*, 2005; Emlen & Nijhout, 2000; Knell, 2009a; Mccullough *et al.*, 2015). Although we did not directly examine the behavioural differences between male morphs in the current study, there is no reason to suspect that these beetles would differ from the well documented behavioural differences observed in other species of dung beetles (Moczek and Emlen, 2000). Behavioural observations indicate that morphs differ in their reproductive strategies, where

‘major’ males with relatively large horns guard tunnels containing females and engage in contests of strength with other males attempting to monopolise females. In contrast, hornless/small horned ‘minor’ males attempt to gain sneak copulations with females by bypassing the guarding male via side tunnels (Moczek and Emlen, 2000). It seems likely that hornless species will have a single reproductive strategy, possibly associated mainly with mate finding and scramble competition (Pomfret and Knell, 2008).

The existence of different male reproductive strategies may act to reduce male reproductive skew as major males are less able to fully exclude rivals using sneak tactics from gaining copulations, thus reducing the effect of ‘good genes’ sexual selection. A comparative study of 16 beetles species from the genus *Onthophagus* found that minor males generally invest more in and have larger testes than major males (Simmons, Emlen and Tomkins, 2007), suggesting increased sperm competitiveness in minor males, which may reduce reproductive skew between minor and majors. However, in *O. taurus* the reproductive success of males from experimental populations containing both major and minor males found that majors, particularly the largest majors, had considerably greater reproductive success than minors even given the alternative tactics employed by the latter (Hunt and Simmons, 2001). In *O. taurus* there is no difference in the investment in testes and ejaculate expenditure between male morphs (Simmons, Tomkins and Hunt, 1999) and given equal opportunity the fertilisation success of minor and major males do not differ (Tomkins and Simmons, 2000). Suggesting that a difference in access to copulations exists and that minors are at least partially excluded from gaining copulations within a competitive environment (Hunt and Simmons, 2001).

Copulations are also unlikely to be guaranteed just by minors gaining access to female tunnels by bypassing guarding males. In three species of *Onthophagus* that have been studied males must perform a courtship display consisting of rapidly tapping the females back with

head and forelimbs before females accept copulation (Kotiaho, Simmons and Tomkins, 2001; Kotiaho, 2002). Females assess males based upon their rate of courtship display, which has been shown to be condition dependent (Kotiaho, Simmons and Tomkins, 2001; Kotiaho, 2002) and in two of the three species studied independent of male morph (Kotiaho, 2002). Furthermore, because ‘sneaks’ are gaining some matings with females it is likely that fertilisation success will be under direct selection from sperm competition. The risk and intensity of sperm competition are expected to differ between male morphs because minors will nearly always be mating with a previously mated female, whereas majors may be able to exclude other rivals from gaining copulations (Simmons, Emlen and Tomkins, 2007). Due to this asymmetry in sperm competition, testes mass in minors has been shown to be condition dependent (Knell and Simmons, 2010).

The condition dependence of courtship rate and testes mass suggest that only males in good condition, irrespective of morph, will successfully sire offspring and provides another mechanism other than contest competition by which sexual selection can act to purge the population of deleterious mutations and promote beneficial alleles (Rowe and Houle, 1996; Lorch *et al.*, 2003; Whitlock and Agrawal, 2009). Furthermore, the existence of different male reproductive strategies may act to increase the effective population size, maintaining genetic diversity and reduce inbreeding caused by increased reproductive skew (Kokko and Brooks, 2003) compared to a system in which all males adopt the same reproductive strategies, in this case hornless males.

We predicted that the scaling relationship between horn length and body size may alter an individuals’ ability to match their phenotype to an altered environment and our results suggest that differences in this scaling relationship may alter a species ability to adapt to altered habitats. Sigmoidal allometries are generally associated with a threshold body size in which males larger than this body size grow horns and those smaller have horn growth

suppressed (Knell, 2009a). This threshold is likely controlled by hormones which are expressed near the end of larval feeding and ‘reprogram’ the development of horns (Emlen & Nijhout, 1999, 2001). In contrast, species with discontinuous allometries, ‘reprogramming’ appears to be more probabilistic occurring across a wider range of body sizes. Furthermore, in the current study minors from species with discontinuous allometries still develop horns with positive allometries, often steeper than those observed in majors: a similar pattern to one recently described in Dynastid beetles (McCullough *et al.*, 2015). The ability of species with continuous sigmoidal allometries to developmentally switch between phenotypes and completely/near completely suppress horn development in smaller males of poor condition may reduce the cost of horn expression and allow increased specialisation of alternative reproductive strategies to be adopted compared to those species with discontinuous allometries in which males in poor condition still invest in horns and possibly suffer increased mortality due to cost associated with horns. This provides a possible explanation to our observation that species with discontinuous allometries show less ability to persist in the most disturbed environments but clearly a more detailed approach is needed to test this hypothesis.

Testes size was found to influence the persistence of species, with model summaries suggesting that species with relatively large testes have a reduced abundance in the most modified habitats. This is contrary to our predictions that species with relatively large testes would have an increased ability to persist in altered habitats, a prediction made because of the relationship between female polyandry and genetic diversity of offspring influencing adaption. Testes size in birds was shown to be positively associated with risk of extinction (Morrow and Pitcher, 2003) and our results suggests a similar effect. The negative association in the current study could potentially be associated with changes in paternal provisioning, which is known to occur in dung beetles (Rasmussen, 1994; Sowig, 1996; Hunt

and Simmons, 1998). In species with increased intensity and risk of sperm competition, the likelihood of offspring being sired by another male is higher, and so paternal provisioning might be reduced, particularly in disturbed habitats where resources may be scarce. Similar findings have been made in birds where the offspring of females which engage in extra-pair copulations often receive less paternal care (Møller, 1991). It is important to note that we don't have much confidence in this particular finding because slopes from each habitat do not significantly differ from zero. Previous studies in *O. taurus* found that testes mass was condition dependent but did not respond to increased sexual selection whereas male strength did (Almbro and Simmons, 2014). Another study in the same species with longer duration of selection did however find a response in testes mass (Simmons and Garcia-Gonzalez, 2008). These results suggest that post-copulatory sexual selection may not be under as strong selection as pre-copulatory sexual selection in dung beetles (Almbro and Simmons, 2014) and against our overall findings of only a small effect of testes size and the large effect in both horn expression and length tends to agree with this.

Against our predictions we find that sexual size dimorphism (SSD), our other estimate of the strength of pre-copulatory sexual selection in a species, has no relationship with species persistence during habitat change. SSD is generally expected in species which engage in contests because larger males will often outcompete smaller males and there is evidence that body size is often a good predictor of contest outcomes in dung beetles (Emlen, 1997; Moczek & Emlen, 2000; Pomfret & Knell, 2006). Male body size in dung beetles may however have an upper limit due to contests taking place in tunnels dug by females. It has been suggested that at larger body size there may be an uncoupling between body size and horn length, possibly an explanation to the observed declines in allometric slopes often seen in the largest males (Pomfret and Knell, 2006). Due to this upper limit on male body size

SDD may not however be a good indicator of the strength of sexual selection in dung beetles which would explain the lack of any effect in the present study.

Here, the analyses with and without a control for phylogenetic relatedness generally show similar findings. We do however find that when controlling for phylogeny particularly in OPP, horn presence/absence and relative horn length are no longer good predictors of species abundance and we no longer detect that possessing horns and having relatively large horns is associated with maintaining species abundance. Distinguishing whether this is caused by reduced power in those models controlling for phylogenetic relatedness or because this is due to more closely related species responding similarly to habitat change is difficult. The majority of species which survive in OPP are groups of closely related species of *Onthophagus* and there is strong and moderate phylogenetic signal in relative horn length and whether a species possesses horns, respectively. This suggests that our findings of pre-copulatory sexual selection increasing persistence within OPP may be due to closely related species having similar ecological responses. We do however find that even with reduced power when controlling for relatedness, that possessing horns and the relative size of those horns is a good predictor of species abundance within moderately modified landscapes (i.e. SLF and HLF). Indicating that our overall interpretation that male contest can drive adaptation, has strong support when habitats are only moderately disturbed.

By estimating the strength of sexual selection that species experience and species persistence and abundance across a gradient of habitat modification, our data suggest that species with increased intensity of male contest competition tend to persist and maintain similar abundances within altered forest habitats. The increased competition in male populations is likely skewing reproductive success in favour of those individuals most suited to the current environmental conditions and driving local adaptation. This is in agreement with a number of theoretical studies which suggest if sexual selection is based upon condition dependent

secondary sexual traits, sexual selection can act to promote good genes, increasing local adaptation and niche width (Proulx, 1999, 2002; Whitlock, 2000; Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016). The lack of a strong effect of post-copulatory sexual selection and SSD suggests that not all aspects of sexual selection have the same costs and benefits to populations as each other and may be trait specific. Understanding species response to anthropogenic driven environmental change is of clear importance and our study suggests that those species with little male-male competition may have reduced ability to adapt, compared to species where males compete for access to copulations.

Chapter 5: Condition dependent expression of horns and testes in altered habitats and the implications to sexual selection

Dung beetles were collected by Dr Eleanor Slade in 2011. Dr Eleanor Slade and Sol Milne helped with dung beetle collections in 2015.

5.1 Abstract

Habitat change has previously been shown to alter the expression of sexually selected traits and influence mating systems. This may have broader implications to the role of sexual selection in adaptation. To test this, dung beetles were sampled from across a gradient of habitat disturbance and the expression of sexually selected traits measured. Comparisons were made between estimates of how well populations were adapted to different habitat types, measured as overall abundance and individuals' condition (mass controlled by body size) and the expression of horns and testes, both previously shown to be important in pre- and post-copulatory sexual selection and to be condition dependent. Here I show that when populations are moved away from their adaptive peaks by habitat change the expression of horn lengths is generally reduced, leading to changes in the proportions of major and minor male morphs within a habitat and potentially altering the relative fitness outcomes of each male reproductive strategy. Contrastingly, when species appear to be reasonably well adapted across all habitats there does not appear to be a difference in the expression of horns. There was good evidence across most species examined that testes mass was strongly affected by male condition, but despite individuals of certain species showing reduced condition in altered habitats, no effect of habitat was found on the expression of testes. These differences between the expression of horns and testes are probably associated with the different life

stages that these traits develop at, which will be influenced in different ways by available resources.

5.2 Introduction

When the environment is altered populations are expected to be moved away from their adaptive peaks. This mismatch between phenotypes and the environment is likely to cause declines in overall population abundance and possibly drive populations to extinction (Burger and Lynch, 1995). Adaptation to new environmental conditions may be enhanced by sexual selection but conversely, the altered costs of expressing sexually selected traits within a novel environment may hinder adaptation and increase extinction risk (Candolin and Heuschele, 2008). Investigating the impacts of environmental change on the expression of sexually selected traits and how mating systems are altered may be useful to furthering our understanding of mechanisms of adaptation and the potential causes of population extinction.

Predictions made by the ‘genic capture’ model of sexual selection (Rowe and Houle, 1996) are that sexually selected traits should show condition dependence and there is high genetic variance in condition. Condition is defined as the amount of available resource which can be allocated to traits which enhance fitness, in this case sexually selected traits. In this broad view, overall condition of an individual will be affected by the majority of an animal’s genome, with any deleterious mutations or non-adaptive alleles reducing the overall pool of available resources (Rowe and Houle, 1996). As resources are finite only those individuals in good condition will be able to express exaggerated traits and the degree of exaggeration should reflect the underlying genetic quality of the bearer. This model of sexual selection can largely explain the maintenance of variation in sexually selected traits despite strong directional selection (Kirkpatrick and Ryan, 1991), because there is a large target for mutation variation for selection to act upon (Rowe and Houle, 1996; Kotiaho, Simmons and Tomkins, 2001; Cotton, Fowler and Pomiankowski, 2004b; Tomkins *et al.*, 2004). Due to reproductive skew caused by sexually selected traits, sexual selection may act to purge

deleterious mutations from a population and also increase adaptation rates (Lorch *et al.*, 2003; Whitlock and Agrawal, 2009; Martínez-Ruiz and Knell, 2016).

Experimental manipulation of resources by limiting nutritional intake is a widely used method for demonstrating the condition dependence of traits (Cotton, Fowler and Pomiankowski, 2004b). This method has demonstrated in a number of species that traits expected to be under directional selection by sexual selection are condition dependent: starved males show reduced courtship rates (Kotiaho, Simmons and Tomkins, 2001), expression of ornaments (David *et al.*, 2000; Cotton, Fowler and Pomiankowski, 2004a) and weaponry (Emlen, 1994). Further evidence comes from the dung beetle, *Onthophagus taurus*, where different male reproductive tactics causes asymmetrical selection on male traits, individuals which engage in male-male competition show condition dependence for strength, whereas males which face increased intensity of sperm competition show condition dependence in testes mass (Knell and Simmons, 2010). Manipulation of resource however, has also been shown to not effect traits known to be under directional selection (Gray & Eckhardt, 2001). Another way to test for condition dependence is to induce fitness reducing mutations through, for example, ionizing radiation or X-ray irradiation (Radwan, 2004; Almbro and Simmons, 2014; Power and Holman, 2015). Almbro and Simmons (2014) found that irradiated *O. taurus* males showed a reduction in both male strength and testes mass, corroborating previous findings that both pre- and post-copulatory sexually selected traits are condition dependent and offer a large target for mutation variation (Knell and Simmons, 2010).

Environmental change may equally cause a reduction in the expression of costly condition dependent sexually selected traits (Kokko and Brooks, 2003). A recent study showed that male collared flycatchers, *Ficedula albicollis*, have reduced the expression of ornamentation over a thirty year period (Evans & Gustafsson, 2017). Increasing spring temperatures over the

study period was associated with a reversal in the selection acting on a sexually selected trait and largely driven by a negative correlation between ornamentation and individual survival. In a second example, sexually dimorphic traits in the newt, *Lissotriton helveticus*, were found to have reduced expression in pine and eucalyptus plantations compared to native forest habitats and it was suggested that differences in ability to acquire resources in altered habitats are likely the reason for this observation (Iglesias-Carrasco *et al.*, 2016). These examples indicate that sexually selected traits may respond both evolutionary and plastically to habitat change.

In addition to affecting traits via condition dependence, environmental change can alter the costs and benefits of expressing sexually selected traits. For example, water eutrophication and habitat complexity alters female choice in three-spined sticklebacks, *Gasterosteus aculeatus*, relaxing selection and causing the expression of sexually selected traits to be detrimental in the altered environment (Candolin, Salesto and Evers, 2007; Heuschele, Salminen and Candolin, 2012). Habitat complexity has also been shown to alter the relative fitness payoffs of males developing into fighter morphs in the bulb mite, *Rhizoglyphus echinopus*. When habitat complexity was increased fewer males develop into fighters due to decreased reproductive success compared to when competing in a simple habitat (Tomkins *et al.*, 2011). Another example of the relative fitness consequences of expressing sexually selected traits being influenced by a change in the environment comes from the introduction of field crickets, *Teleogryllus oceanicus*, to Hawaiian Islands. Populations faced a new selective pressure of an acoustically orienting parasitoid fly. Males which call to attract females have been selected against and a large proportion of the male population have now lost the ability to call and adopt a silent satellite strategy (Zuk, Rotenberry and Tinghitella, 2006).

Whether the expression of sexually selected traits are associated with the overall health of a population has largely been ignored. A limited number of field studies have investigated how variation in sexually selected traits and behaviours vary between habitats (Vergara *et al.*, 2012; Iglesias-Carrasco *et al.*, 2016) but have not included estimates of other aspects of population fitness i.e. population size or reproductive output. Anthropogenically altered habitats provide a good way to test these ideas. To address these questions I examine the expression of sexually selected traits of a number of dung beetle species that are likely to be ecologically important in tropical forest in Sabah, Malaysia. Dung beetles were sampled across a disturbance gradient of tropical rainforest, ranging from old-growth rainforest to oil palm plantation. Within the altered habitats a number of abiotic environmental factors have been shown to be altered including air temperature, soil temperature and humidity. In oil palm plantation day time air temperatures have been shown to be approximately 6°C warmer, peak soil temperature approximately 3°C warmer and relative humidity reduced by about 20% compared to forest habitats (Hardwick *et al.*, 2015). The diversity of mammals is generally reduced as land use intensification increases (Flynn *et al.*, 2009), and this same pattern has been shown within the study site investigated here (Wearn *et al.*, 2016). Due to dung beetles being primarily feeders on mammalian dung this reduction in mammalian diversity may also correspond to an overall decrease in available resources.

Specifically I investigate whether the scaling relationship between horn length and body size, if the proportion of each male morph and the relative testes mass of each species differ across the gradient of habitat disturbance. Furthermore, I estimate how well a population was adapted to each habitat type using both the overall abundance of each species and the condition of males, calculated as total body mass controlled for by body size. The expression of sexually selected traits are discussed in relation to overall species abundance and condition. I predict that in habitats where species are found in high abundance they will also

be in relatively good condition and when abundance is reduced there will be a corresponding reduction in the condition of individuals. By taking into account both abundance and condition datasets the habitat which species are most well adapted to will be highlighted. I predict the expression of sexually selected traits will be altered if populations differ in how well they adapted to each habitat. Within each species I predict to observe relatively large horns for a given body size, a greater proportion of males expressing horns and therefore a greater proportion of major males and increased testes mass in the habitat each species is most well adapted to compared to habitats species are less well adapted to. Furthermore, due to the asymmetry in sperm competition I predict minor males to have relatively large testes and due to condition dependence of testes in minor males the effect of habitat on testes mass will be greater in minor males compared to major males.

5.3 Methods

5.3.1 Dung beetle collection and trait measurement

These data presented here are from the same individuals in chapter 4. Dung beetle collections were carried out across a habitat disturbance gradient, this gradient included old-growth rainforest (OGF), selectively logged forest (SLF), heavily logged forest (HLF) and oil palm plantation (OPP). For details on trapping and trait measurement see sections 4.3.1 and 4.3.2, respectively.

5.3.2 Species selection

I selected six dung beetles to investigate whether the expression of sexually selected traits differs between habitats. The dung beetle species chosen were *Catharsius dayacus*, *Catharsius renaudpauliani*, *Proagoderus watanabei*, *Onthophagus obscurior*, *Onthophagus cervicapra* and *Onthophagus negroboscior*.

C. dayacus and *C. renaudpauliani* were chosen because the presence of *C. dayacus* has previously been shown to be positively associated with ecosystems services and functioning (Slade *et al.*, 2007; Slade, Mann and Lewis, 2011). Furthermore, *C. dayacus* and *C. renaudpauliani* are likely to have similar niches, but with the former being a forest specialist and the latter a disturbed habitat specialist with an overlapping distribution in moderately disturbed sites (Darren Mann, personal communication).

P. watanabei was selected because its relatively large body size makes it a candidate for being ecologically important (Larsen, Williams and Kremen, 2005; Slade *et al.*, 2007; Slade, Mann and Lewis, 2011). Furthermore, *P. watanabei* males express a pair of long curved head horns and a single pronotum horn (Figure 5.1), which are the largest horns relative to body size found within this community of dung beetles (see Table 4.1). Previous work examining the allometric scaling relationships between horn structures and body size in *P. watanabei*

has indicated that expression of both horn structures is positively correlated (Moczek, Brühl and Krell, 2004), indicating that overall horn length in this species possibly reflects overall male quality, making it a potentially interesting species to investigate.

O. obscurior and *O. cervicapra* were selected due to their overall high abundance, being the first and fourth most abundant out of all species collected (see Table 4.1). *O. negroboscuro* although having relatively low abundance was also included as these three *Onthophagus* species are closely related (see Figure 4.1), but *O. negroboscuro* is only found in OGF and SLF, compared to *O. obscurior* and *O. cervicapra* which are found across all habitat types (see Table 4.1), suggesting *O. negroboscuro* could be a good candidate as an indicator species.

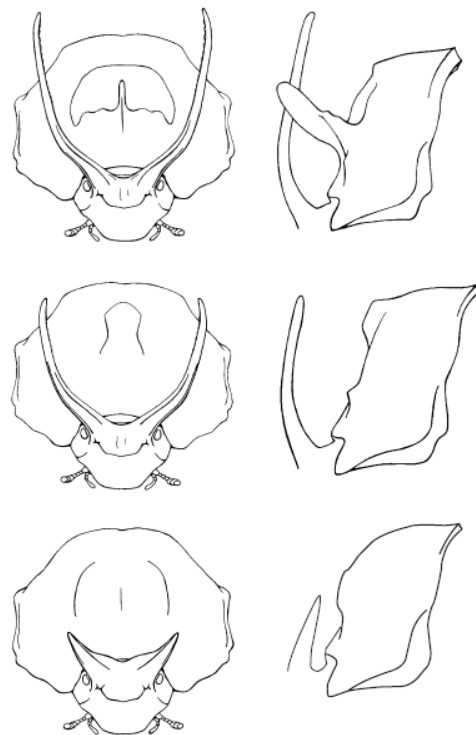


Figure 5.1: *P. watanabei* male head and pronotum morphology: top: male with large head horns and pronotum horn; middle: male with large head horns but no pronotum horn; bottom: male with small head horns and no pronotum horn. Drawing by Beth Archie from (Moczek, Brühl and Krell, 2004).

5.3.3 Statistical analysis

All data was amalgamated to the level of replicate (see section 4.3.1). All statistical analysis was carried out in R version 3.3.0 (R Development Core Team, 2016). Unless stated model selection was performed by removal of non-significant terms until the minimal adequate model was reached. Final models were checked for heteroscedasticity of residuals and response variables log transformed where appropriate to improve model fitting.

The abundance of each species was compared across habitats by fitting simple general linear models with quasipoisson error structures to account for overdispersion. In a number of species no individuals were found in certain habitat types, in these cases these data were removed and models refitted.

The allometric relationship between horn length and body size was determined from all individuals following guidelines set out in Knell (2009a) and described in full in section 4.3.4.2. *P. watanabei*, *C. dayacus* and *C. renaudpauliani* possess both head and pronotum horns, visual inspection of log-log scatterplots were made of pronotum horn length-body size allometry and compared with head horn length-body size scatterplots. Within *C. dayacus* and *C. renaudpauliani* the majority of males would be assigned to the same morph whether this division was made using head or pronotum horns, therefore only head horn data is presented. In *P. watanabei* by contrast, visual inspection of both pronotum and head horn scatterplots indicated a different scaling relationship between the two horn types and that a more complex scaling relationship of overall horn expression and body size may exist in this species, with three rather than two male morphs. This was investigated by assigning a binary factor of pronotum horn presence or absence and incorporating this into the log-log scatterplot of head horn-body size.

Whether the allometric relationship was affected by habitat was tested in two ways based on what the overall allometric relationship was. In *C. dayacus*, *C. renaudpauliani* and *P. watanabei* a discontinuous allometric relationship best described the scaling relationship between horns and body size and males were assigned a morph based on the frequency distribution of head horn to body size ratio. General linear mixed effects models were fitted using *lme4* (Bates *et al.*, 2015) with log horn length as the response variable and log body size, male morph and habitat type including all two way interaction terms as explanatory variables. Year of collection was included as a fixed effect and replicate as a random effect.

O. obscurior, *O. cervicapra* and *O. negroboscuro* all showed sigmoidal allometries. The body size switchpoint between minor and major was estimated by removing the asymptotic major males (where the slope begins to decline back to zero) and fitting a switchpoint model using the R package *segmented* (Muggeo, 2003), males larger than this switchpoint being assigned as majors and those smaller as minors. Initially changes in allometry were compared by fitting switchpoint models to individuals from each habitat type and comparing whether there was overlap in the standard errors estimated from *segmented* between habitats to indicate differences. This approach gave questionable results due to low numbers of minors within certain habitat types. Generalised additive models (GAM) were then fitted to full horn length-body size data for each species. Full models were fitted with log horn length as the explanatory variable and habitat, year and log body size with a spline smoother and habitat interaction as explanatory variables, the full model was then simplified by removing terms one at a time and goodness of fit compared using AIC scores. This approach estimates whether any differences in the overall scaling relationship exists.

After the assignment of male morph (as above) the proportion of males which were classified as majors were compared across habitat types by fitting generalised linear mixed effects models (GLMMs) with binomial error structure with habitat and year as explanatory

variables and replicate as a random effect. *P. watanabei* was assigned three morphs; therefore three GLMMs were fitted to compare the proportion of each morph across habitat types.

GLMMs were fitted to testes mass data with total mass, horn length, male morph and habitat type including all two way interactions as explanatory variables. Body size was fitted as a fixed effect and replicate as a random effect. Following inspection of residuals, log transformation of testes mass of *C. renaudpauliani* and *O. negroboscior* was carried out and models refitted.

The condition of males was assessed by fitting GLMMs to total mass data with habitat and male morph including their two-way interaction as explanatory variables. Body size was fitted as a fixed effect and replicate as a random effect. Following inspection of residuals, log transformation of total mass was carried out to improve heteroscedasticity and models refitted.

5.4 Results

5.4.1 Abundance and condition

5.4.1.1 *Catharsius* spp.

There was a significant effect of habitat on the abundance of *C. dayacus* ($\chi^2 = 10.65$, $df = 1$, $p = 0.004$; Figure 5.2a). The highest abundance of *C. dayacus* was observed in OGF, with fewer individuals found moving along the disturbance gradient and no individuals found within OPP. The model assessing male *C. dayacus* condition (body mass controlled for by body size) retained the two-way interaction between habitat and male morph in the minimal adequate model ($\chi^2 = 6.07$, $df = 1$, $p = 0.048$; Figure 5.2b). Within the least disturbed habitats i.e. OGF and SLF major males have increased relative mass (i.e. condition) compared to minors, but this difference is no longer observed in HLF where each male morph has similar relative masses at any given body size.

The abundance of *C. renaudpauliani* significantly differed between habitats ($\chi^2 = 10.65$, $df = 1$, $p = 0.004$; Figure 5.3a). The number of *C. renaudpauliani* found in each trap was similar in HLF and OPP, with significantly fewer individuals trapped in SLF and none in OGF.

There was a close to significant main effect of habitat on the condition of *C. renaudpauliani* males ($\chi^2 = 5.47$, $df = 1$, $p = 0.065$), this suggests that males in SLF are in poorer condition, but with a small sample size not much confidence can be had in this pattern. Overall, major males in this species tended to have increased mass when controlled for body size than minors, i.e. majors are on average in better condition than minors ($\chi^2 = 11.2$, $df = 1$, $p < 0.001$; Figure 5.3b).

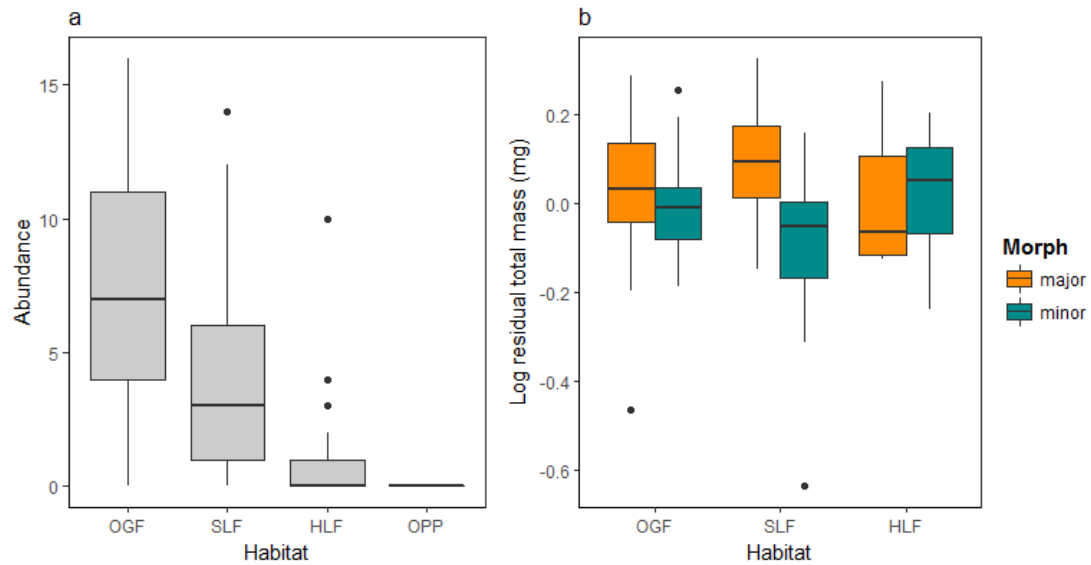


Figure 5.2: (a) Abundance of *C. dayacus* per trap and (b) condition (mass controlled for body size) of *C. dayacus* major (orange) and minor (green) males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation).

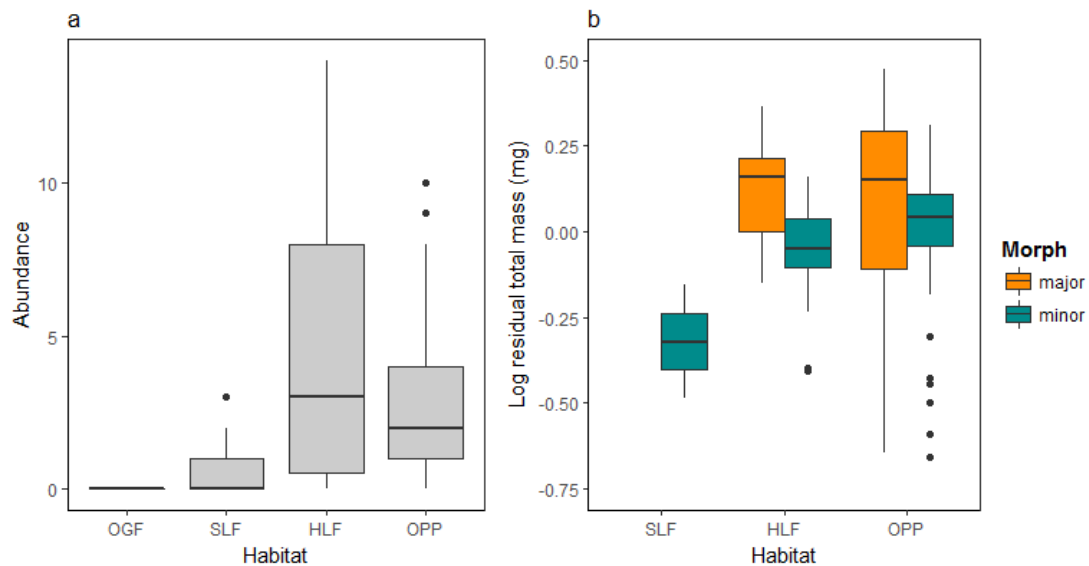


Figure 5.3: (a) Abundance of *C. renaudpauliani* per trap and (b) condition (mass controlled for body size) of *C. renaudpauliani* major (orange) and minor (green) males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation).

5.4.1.2 *Proagoderus watanabei*

The abundance of *P. watanabei* was found to differ between habitats ($\chi^2 = 19.35$, $df = 1$, $p < 0.001$; Figure 5.4a). The highest abundance per trap was found in HLF, significantly fewer individuals found in SLF and OPP, with the lowest abundance observed in OGF. The model assessing the condition (mass controlled by body size) of male *P. watanabei* retained significant main effects of habitat type ($\chi^2 = 16.41$, $df = 1$, $p < 0.001$) and morph ($\chi^2 = 8.35$, $df = 1$, $p = 0.015$). Inspection of figure 5.4b and the model summary indicates that gamma males have significantly lower mass after controlling for body size than both beta and alpha males (see below for description of morphs). Additionally, the main effect of habitat indicates that males found in HLF and OPP are in better condition than males found in OGF and SLF.

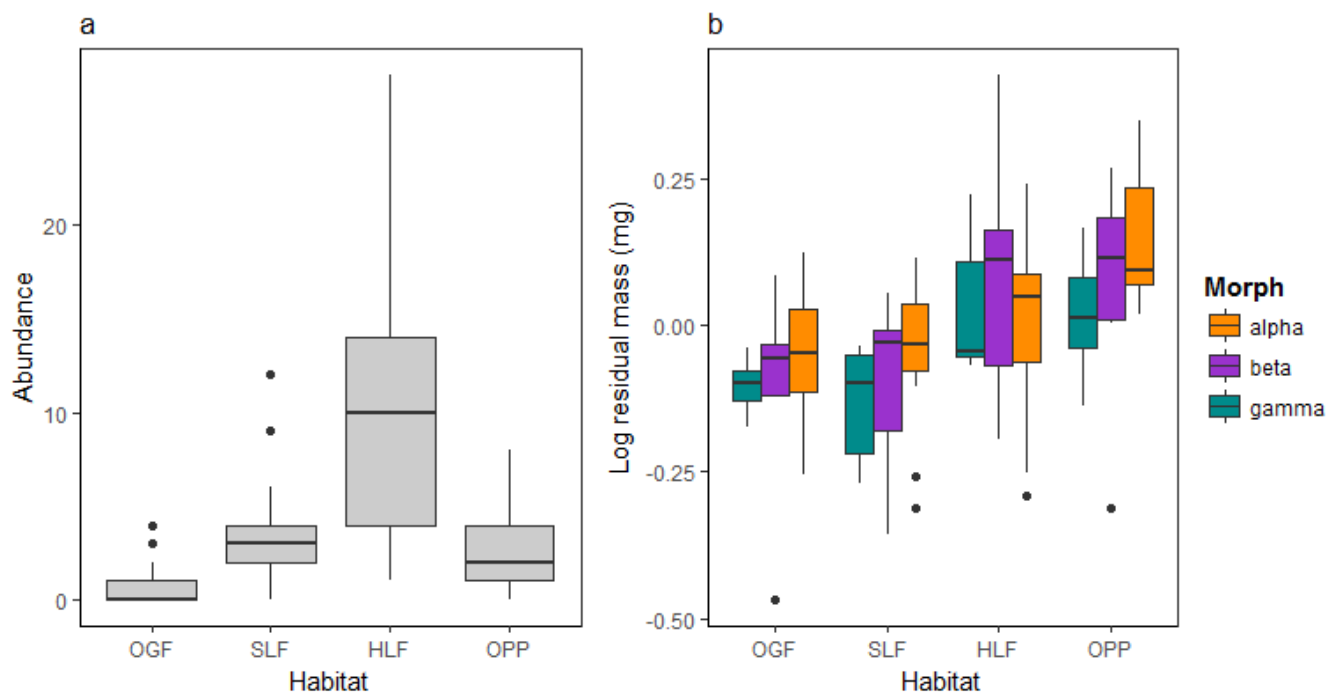


Figure 5.4: (a) Abundance of *P. watanabei* per trap and (b) condition (mass controlled for body size) of *P. watanabei* alpha (orange), beta (purple) and gamma (green) males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation).

5.4.1.3 *Onthophagus* spp.

The abundance of *O. obscurior* was found to differ between habitats, with lower abundance found in SLF compared to all other habitat types, which all maintained similar abundance ($\chi^2 = 10.38$, $df = 1$, $p = 0.016$; Figure 5.5a). The condition (mass controlled for body size) of major and minor males did not differ ($\chi^2 = 3.18$, $df = 1$, $p = 0.075$), there was however a significant effect of habitat with individuals found in SLF having relatively low mass for their given body size compared to all other habitats ($\chi^2 = 13.332$, $df = 1$, $p = 0.004$; Figure 5.5b).

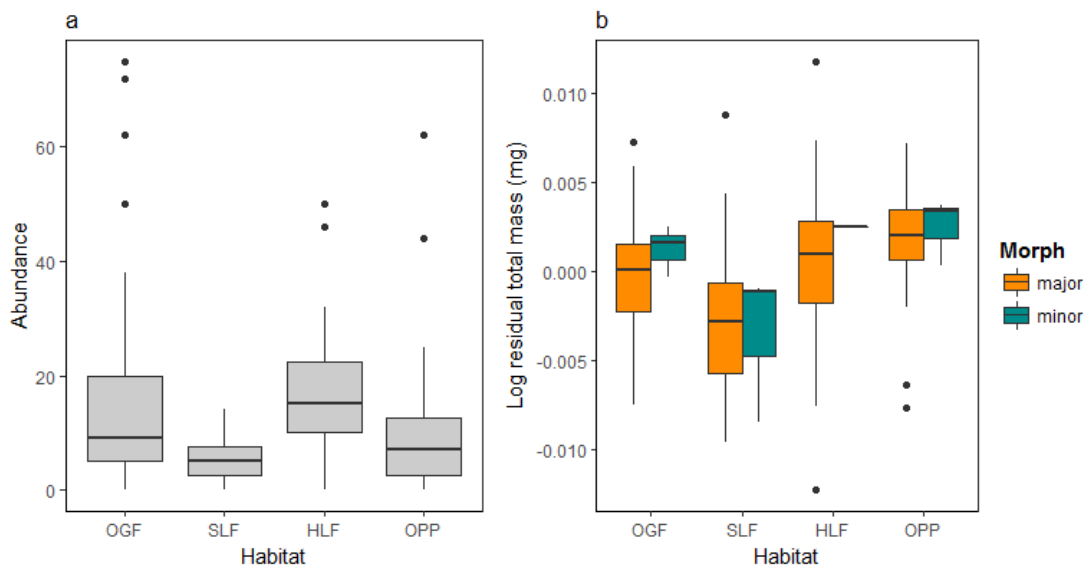


Figure 5.5: (a) Abundance of *O. obscurior* per trap and (b) condition (mass controlled for body size) of *O. obscurior* major (orange) and minor (green) males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation).

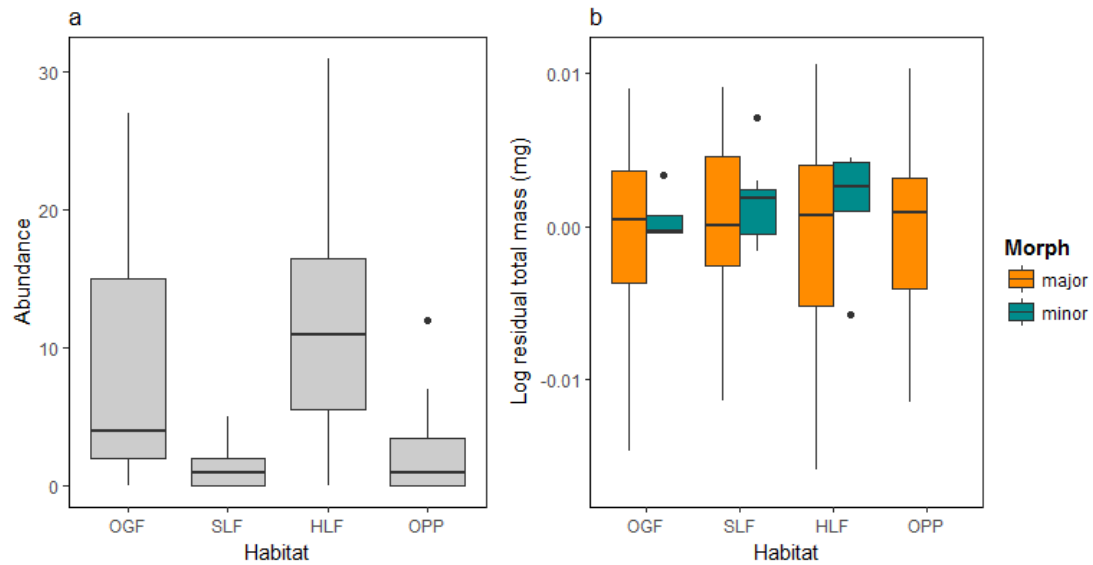


Figure 5.6: (a) Abundance of *O. cervicapra* per trap and (b) condition (mass controlled for body size) of *O. cervicapra* major (orange) and minor (green) males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation).

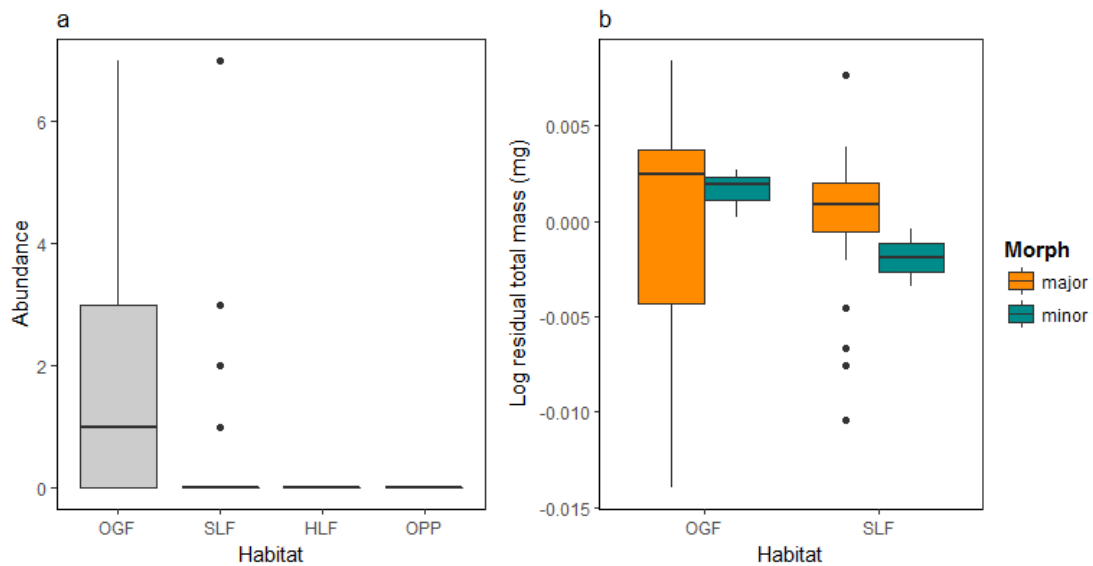


Figure 5.7: (a) Abundance of *O. negroboscuro* per trap and (b) condition (mass controlled for body size) of *O. negroboscuro* major (orange) and minor (green) males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation).

O. cervicapra abundance was found to be reduced in both SLF and OPP, but maintained similar abundances in OGF and HLF ($\chi^2 = 20.19$, $df = 1$, $p < 0.001$; Figure 5.6a). There was no difference in the total mass of males when body size was controlled for between habitats ($\chi^2 = 0.47$, $df = 1$, $p = 0.923$) or morphs ($\chi^2 = 2.26$, $df = 1$, $p = 0.133$).

When the habitats *O. negroboscuro* were not found in (i.e. HLF and OPP) were removed from the dataset there was non-significant effect of habitat on abundance, it is however very close significant, suggesting that fewer individuals were found in SLF compared to OGF ($\chi^2 = 3.82$, $df = 1$, $p = 0.0502$; Figure 5.7a). The mass of male *O. negroboscuro* did not differ between habitats after body size was controlled for ($\chi^2 = 0.03$, $df = 1$, $p = 0.871$) or morphs ($\chi^2 = 0.02$, $df = 1$, $p = 0.879$).

5.4.1.4 Summary and interpretation of abundance and condition data

Overall, abundance and condition data largely support each other and suggest similar findings (Table 5.1). Leading to an interpretation that *C. dayacus* is most well adapted to OGF and SLF, but also persists within HLF. In contrast, *C. renaudpauliani* is most well adapted to HLF and OPP, but persists within SLF at low abundance. *P. watanabei* are found across all habitats; however abundance and condition data are interpreted as *P. watanabei* being most well adapted to HLF and least well adapted to OGF. Similarly, *O. obscurior* and *O. cervicapra* are found across all habitats in the disturbance gradient. Both abundance and condition data indicate that *O. obscurior* is well adapted to OGF, HLF and OPP. *O. cervicapra* abundance and condition data do not entirely align and those data from SLF and OPP show reduced abundance but that condition is maintained similar to OGF across all sites. Interpretation of these data is that *O. cervicapra* is likely to be well adapted to OGF, SLF and HLF, and persisting within OPP at low abundance. *O. negroboscuro* are not found

in the most disturbed habitats (HLF and OPP). In those habitats in which *O. negroboscuro* is found abundance and condition data suggest *O. negroboscuro* is most well adapted to OGF and can persist within SLF.

Table 5.1: Summary of abundance and condition data of the six species investigated.

Habitat/s (OGF, SLF, HLF, OPP) in bold indicate the habitat in which each species has the highest abundance and greatest condition; multiple habitats in bold indicate that these are statistically indistinguishable from each other. The interpretation column indicates which habitats each species are likely to be most well adapted based on both datasets. An asterisk indicates some degree of ambiguity and that these habitats may or may not be bold.

Species	Abundance data	Condition data	Interpretation
<i>C. dayacus</i>	OGF,SLF , HLF, ---	OGF, SLF, HLF* , ---	OGF, SLF , HLF, ---
<i>C. renaudpauliani</i>	---, SLF, HLF, OPP	---, SLF* , HLF, OPP	---, SLF, HLF, OPP
<i>P. watanabei</i>	OGF, SLF, HLF, OPP	OGF, SLF, HLF, OPP	OGF, SLF HLF, OPP
<i>O. obscurior</i>	OGF, SLF, HLF, OPP	OGF, SLF, HLF, OPP	OGF, SLF* , HLF, OPP
<i>O. cervicapra</i>	OGF, SLF, HLF, OPP	OGF, SLF, HLF, OPP	OGF, SLF, HLF, OPP*
<i>O. negroboscuro</i>	OGF, SLF* , ---, ---	OGF, SLF , ---, ---	OGF, SLF , ---, ---

5.4.2 Expression of sexually selected traits

5.4.2.1 *Catharsius* spp.

The model assessing differences in the allometric relationship between horn length and body size in *C. dayacus* retained the main effect of habitat ($\chi^2 = 8.24$, $df = 1$, $p = 0.016$; Figure 5.8). Individuals collected from HLF on average have relatively small head horns compared to individuals collected from OGF and SLF. The main effect of year of collection was significant ($\chi^2 = 8.19$, $df = 1$, $p = 0.004$) and indicates that in 2015 individuals tended to have relatively small head horns compared to 2011 individuals.

The proportion of male morphs collected differed between habitat types in *C. dayacus* ($\chi^2 = 9.14$, $df = 1$, $p = 0.01$; Figure 5.9). A significantly higher proportion males were classified as majors in OGF (66%) compared to HLF and SLF (48% and 42%, respectively). There was no difference between years in the proportion of males morphs collected ($\chi^2 = 1.39$, $df = 1$, $p = 0.239$).

None of the variation in relative testes mass of *C. dayacus* males was explained by any of the explanatory variable used in the model. Total body mass was non-significant ($\chi^2 = 2.94$, $df = 1$, $p = 0.086$), but indicates that condition may be associated with expression of testes.

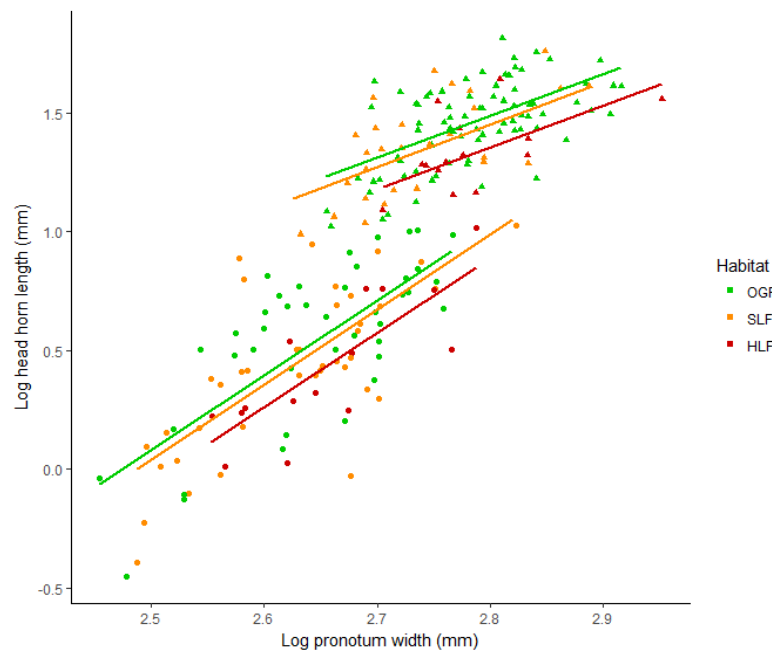


Figure 5.8: Allometric relationships between horn length and body size of *C. dayacus* in different habitats: old growth forest (OGF: green), selectively logged forest (SLF: orange) and heavily logged forest (HLF: red). Circles indicate minor males and triangles major males. The effect of year is omitted from the figure for clarity, if plotted the intercepts of lines from 2015 would be lower.

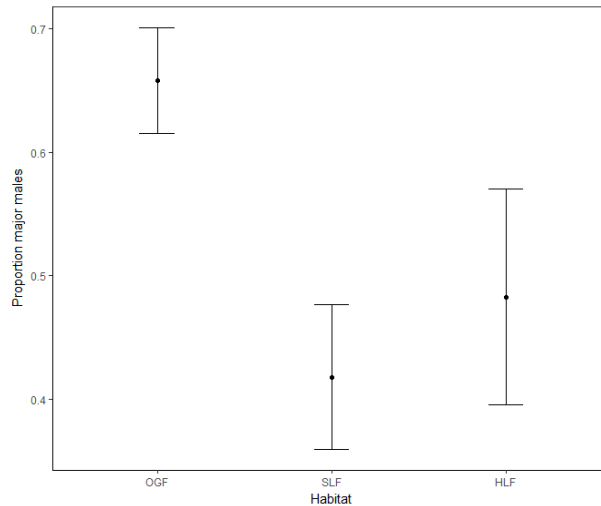


Figure 5.9: Proportion of *C. dayacus* males classified as majors in each habitat type (OGF = old growth forest, SLF = selectively logged forest and HLF = heavily logged forest).

There was no effect of habitat on the allometric relationship between horn length and body size in *C. renaudpauliani* ($\chi^2 = 1.36$, $df = 1$, $p = 0.506$; Figure 5.10). Year of collection was significant, ($\chi^2 = 11.32$, $df = 1$, $p < 0.001$) and indicates that individuals collected in 2015 had relatively small horns compared to those collected in 2011.

There was no effect of habitat type on the proportion of *C. renaudpauliani* male morphs collected ($\chi^2 = 2.01$, $df = 1$, $p = 0.365$), neither was proportion of male morphs effected by year of collection ($\chi^2 = 0.05$, $df = 1$, $p = 0.830$). Inspection of figure 5.4b suggests that the proportion of major males is reduced in SLF but there were very few individuals found in SLF and with a small sample size this pattern was non-significant.

The model assessing *C. renaudpauliani* testes mass retained two two-way interactions between horn length and male morph ($\chi^2 = 7.53$, $df = 1$, $p = 0.006$) and horn length and total mass ($\chi^2 = 6.56$, $df = 1$, $p = 0.01$). There was no effect of habitat on relative testes mass ($\chi^2 = 2.28$, $df = 1$, $p = 0.320$). These interaction terms predicting testes mass in *C. renaudpauliani*

are rather complex, inspection of figure 5.12 shows that when testes mass is controlled for by body size and total mass minor male testes mass is negatively associated with horn length, whereas in major males horn length is not associated with testes mass. The model summary also indicates that males with increased total mass when body size is controlled for have increased testes mass.

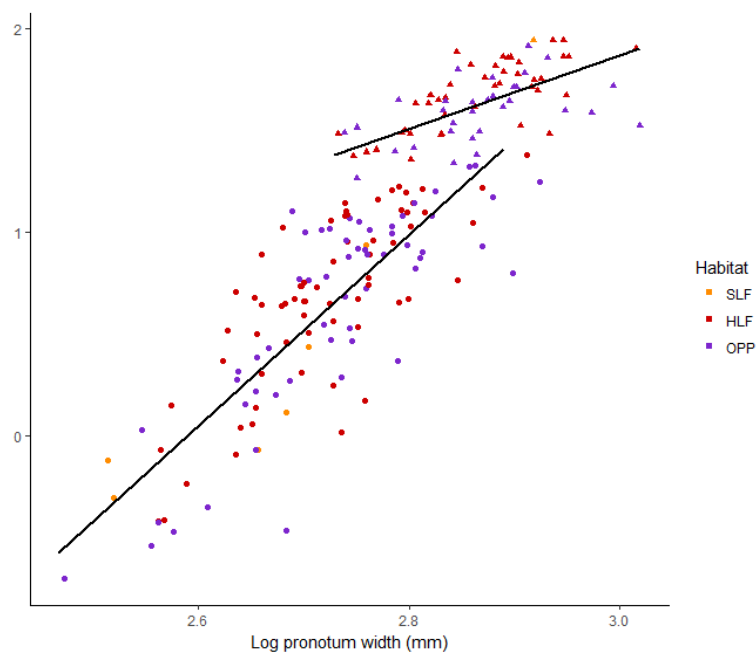


Figure 5.10: Allometric relationships between horn length and body size of *C.*

renaudpauliani in different habitats: selectively logged forest (SLF: orange), heavily logged forest (HLF: red) and oil palm plantation (OPP: purple). Circles indicate minor males and triangles major males. The effect of year is omitted from the figure for clarity, if plotted the intercepts of lines from 2015 would be lower.

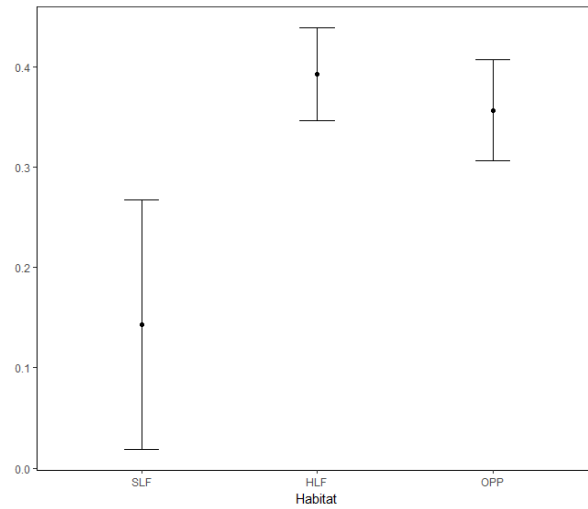


Figure 5.11: Proportion of *C. renaudpauliani* males classified as majors in each habitat type (SLF = selectively logged forest, HLF = heavily logged forest and oil palm plantation = OPP).

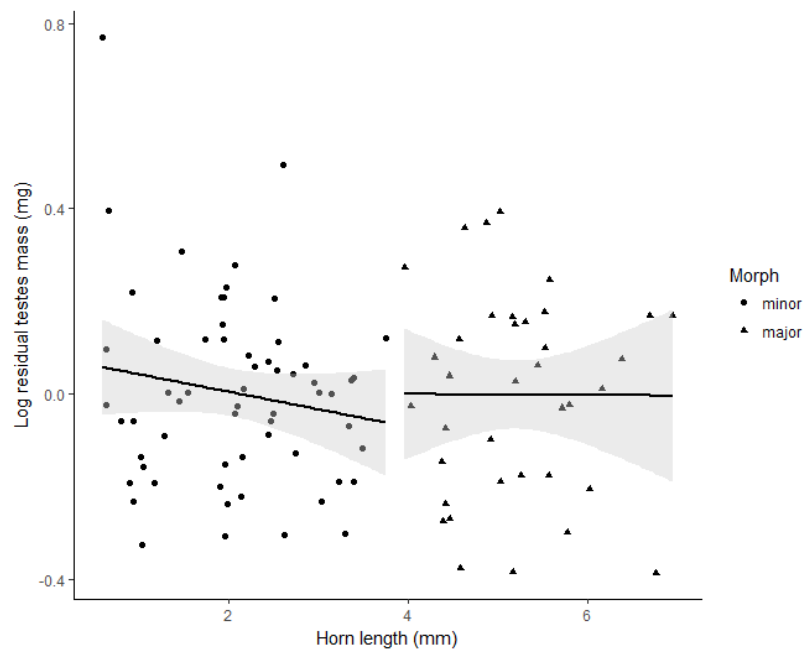


Figure 5.12: Testes mass of *C. renaudpauliani* after the removal of the effects of body size and total mass against horn length. Males classified as minors and majors are indicated by circles and triangles, respectively.

5.4.2.2 *Proagoderus watanabei*

When plotting log-log scatterplot of head horn length against body size and taking into account the binary expression of pronotum horns (Figure 5.13a), there is evidence that three males morphs exist (Figure 5.13b). There are males which have relatively small head horns and no pronotum horn (gamma), males with relatively large head horns but no pronotum horn (beta) and the largest males, which have relatively large head horns and express a pronotum horn (alpha), see figure 5.1 (alpha: top; beta: middle; gamma: bottom). Further inspection of the frequency distribution of the ratio of head horn length to body size (Knell, 2009a) also shows some evidence the three morphs exist based upon head horns alone (Figure 5.13c). Based only upon this frequency distribution the vast majority of males can be categorised as these three morphs (Figure 5.13d), and there are only a small number of individuals which are not easily classified as beta or alpha. For simplicity the classification of the three male morphs is based upon head horns only in all further analysis as shown in figure 5.13d.

The model assessing whether the allometric relationship between head horn and body size differed between habitats retained the two-way interaction between morph and habitat type ($\chi^2 = 20.37$, $df = 1$, $p = 0.002$; Figure 5.14). The two-way interaction is caused by gamma males having relatively small horns in OGF, there is no difference in the scaling relationship between gamma, beta and alpha males in the different habitat types.

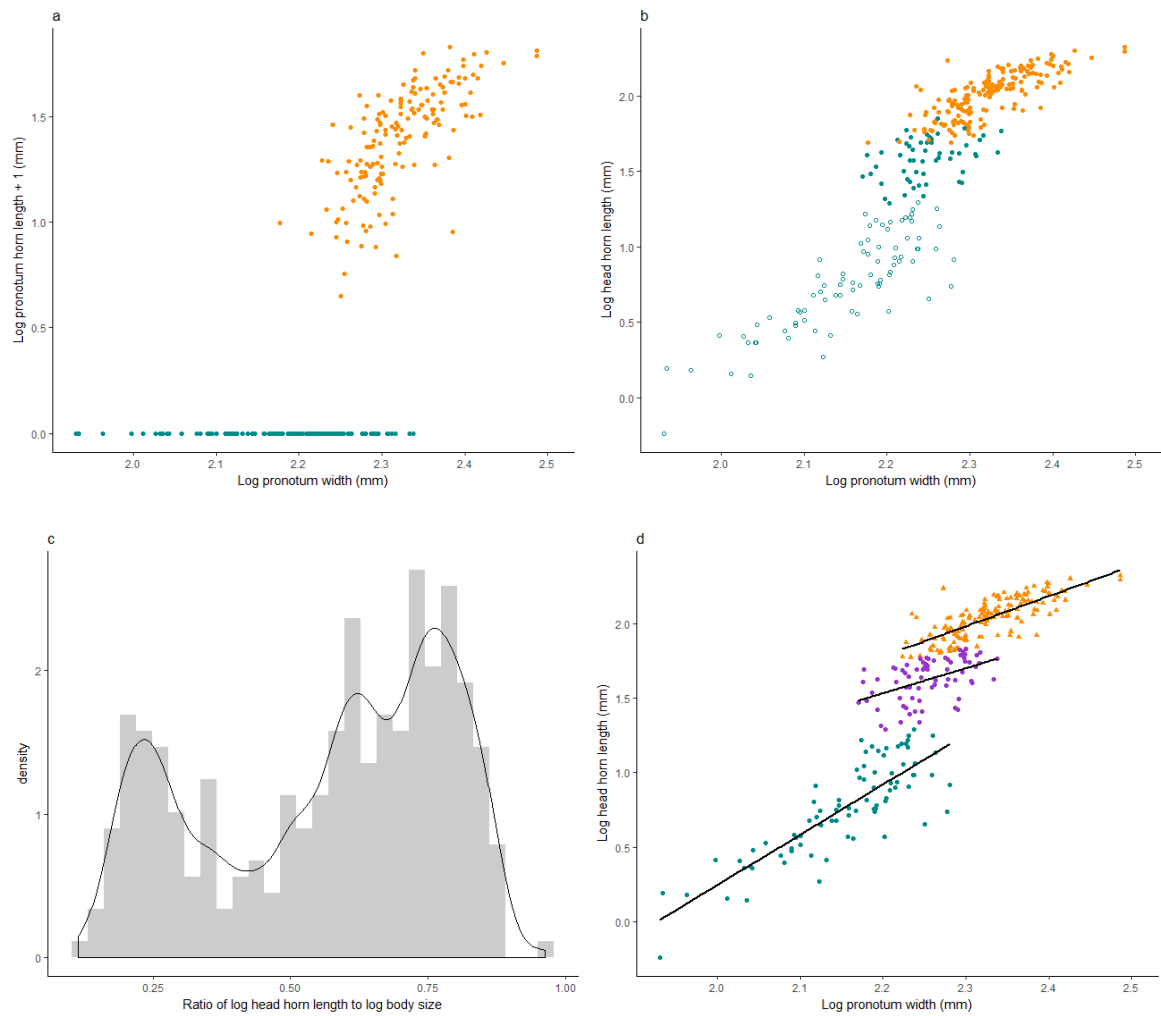


Figure 5.13: *P. watanabei* (a) log-log scatter plot of pronotum horn against body size (green: no pronotum horn; orange: pronotum horn); (b) log-log scatter plot of head horn against body size (green: no pronotum horn; orange: pronotum horn, hollow: minor, based on two male morphs; solid: major, based on two male morphs); (c) histogram of frequency distribution of the ratio of head horn against body size with density line; (d) three male morphs based on both minor and major head horns while including the binary presence/absence of a pronotum horn (gamma: green circles; beta: purple circles; alpha: orange triangles).

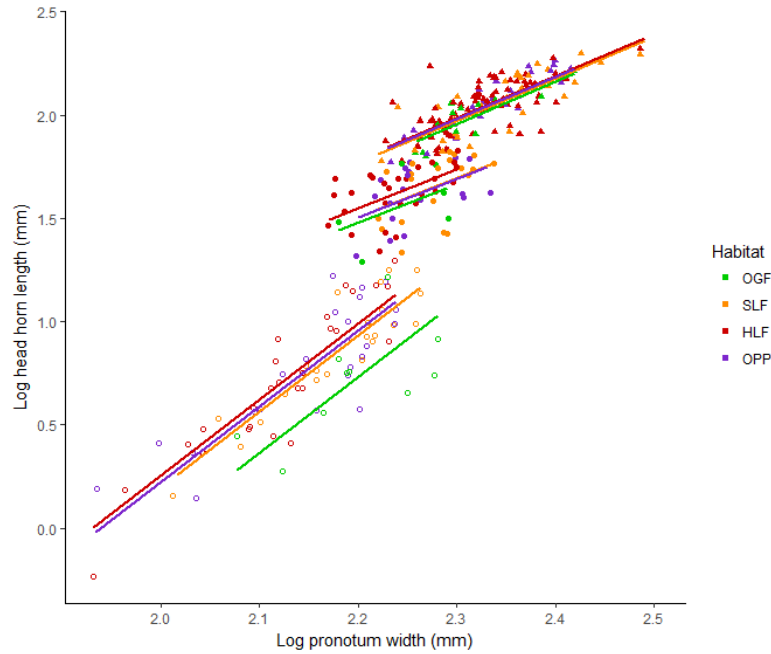


Figure 5.14: Allometric relationships of horn length and body size of *P. watanabei* showing the effect of habitat on this relationship, old growth forest (green), selectively logged forest (orange), heavily logged forest (red) and oil palm plantation (purple). Hollow circle indicate gamma males, solid circles beta males and solid triangles alpha males.

Models were fitted to assess whether the proportion of each *P. watanabei* morph differed between habitats. When comparing the proportion of alpha males there was a significant main effect of habitat type ($\chi^2 = 8.56$, $df = 1$, $p = 0.036$; Figure 5.10), a greater proportion of males found in HLF are classified as majors compared to other habitat type. There were no differences in the proportion of beta and gamma found across habitats (beta: $\chi^2 = 1.87$, $df = 1$, $p = 0.6$; gamma: $\chi^2 = 5.5$, $df = 1$, $p = 0.139$; Figure 5.10). In all models there was no effect of year.

There was no effect of habitat on testes mass within *P. watanabei* ($\chi^2 = 4.69$, $df = 1$, $p = 0.196$). The model did retain the main effect of mass ($\chi^2 = 23.04$, $df = 1$, $p < 0.001$) and the

two-way interaction term between morph and horn length ($\chi^2 = 6.3$, $df = 1$, $p = 0.043$; Figure 5.16). The model estimates indicate that increased relative body mass is positively associated with testes mass. The two-way interaction is mainly driven by a negative association between horn length and testes mass after controlling for mass and body size in beta males. There was no relationship between horn length and testes mass in alpha males and a non-significant positive association in gamma males between horn length and testes mass (Figure 5.16).

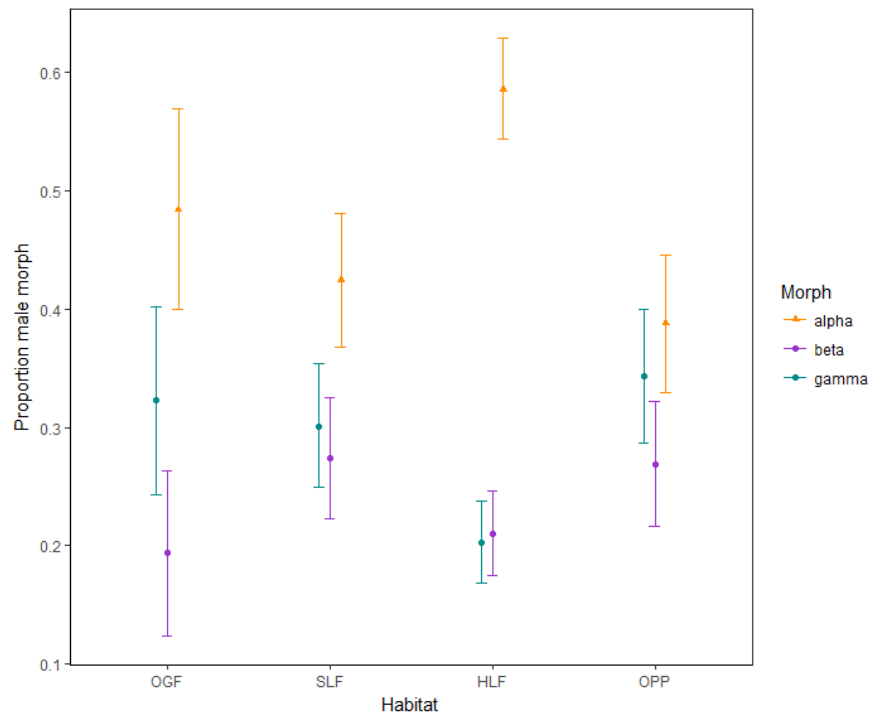


Figure 5.15: Proportion male morphs (alpha: orange triangle; beta: purple circle; gamma: green circle) of *P. watanabei* found in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation). Error bars indicate standard error.

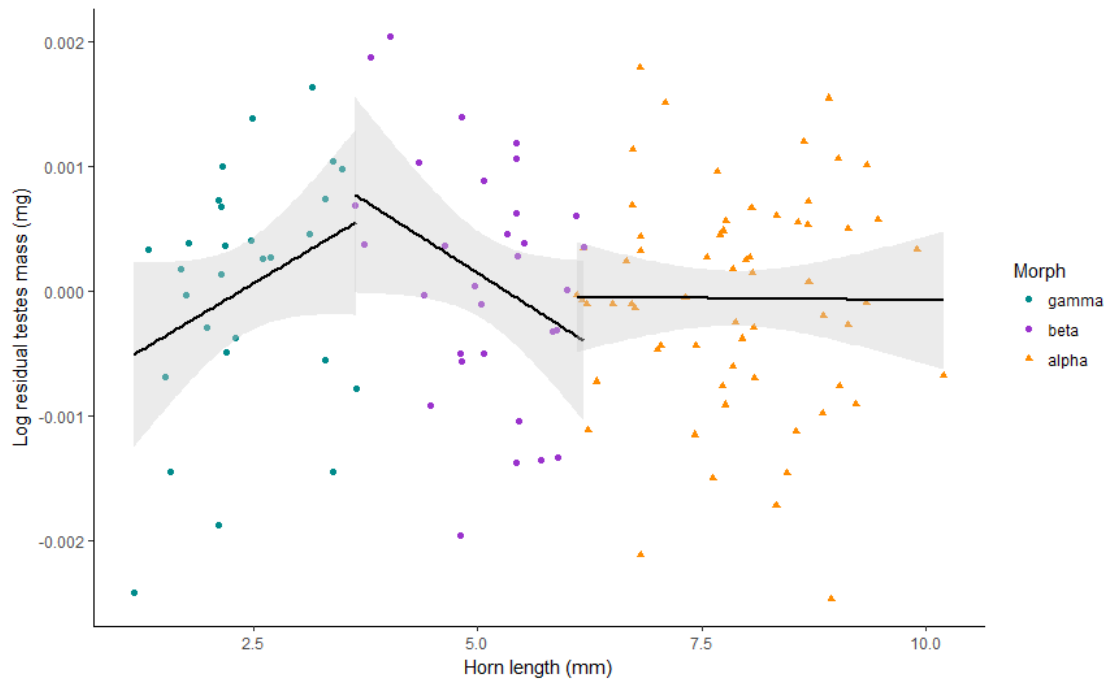


Figure 5.16: Testes mass of *P. watanabei* after the removal of the effects of body size and total mass against horn length. Males classified as alpha males are indicated by orange triangles, beta males by purple circles and gamma males green circles.

5.4.2.3 *Onthophagus* spp.

All *Onthophagus* species in the current study showed sigmoidal allometric relationships between horn length and body size (Figure 5.17a-c). By removing the asymptotic major males the switchpoint body sizes between minors and majors were determined as (log pronotum width (mm) \pm SE): *O. obscurior*: 1.183 ± 0.008 ; *O. cervicapra*: 1.262 ± 0.010 ; *O. negroboscior*: 1.204 ± 0.014 ; figure 5.18a-c. All males larger than this switchpoint are classified as majors and those smaller classified as minors. Comparison of the AIC scores of GAMs fitted to each species found no differences in the allometry of any species between habitats or years. In all cases the models containing only the body size smoother were the best fitting models (Table 5.2).

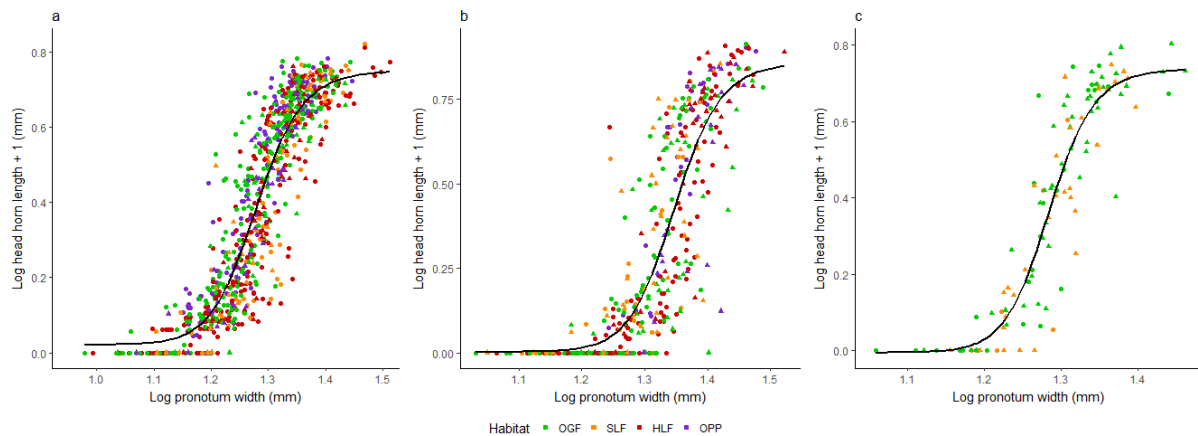


Figure 5.17: Allometric relationships between horn length and body size of (a) *O. obscurior*, (b) *O. cervicapra* and (c) *O. negroboscuro*. Habitat is indicated by colour (OGF: green; SLF: orange; HLF: red; and OPP: purple) and year indicated by point shape (2011: circle; 2015: triangle).

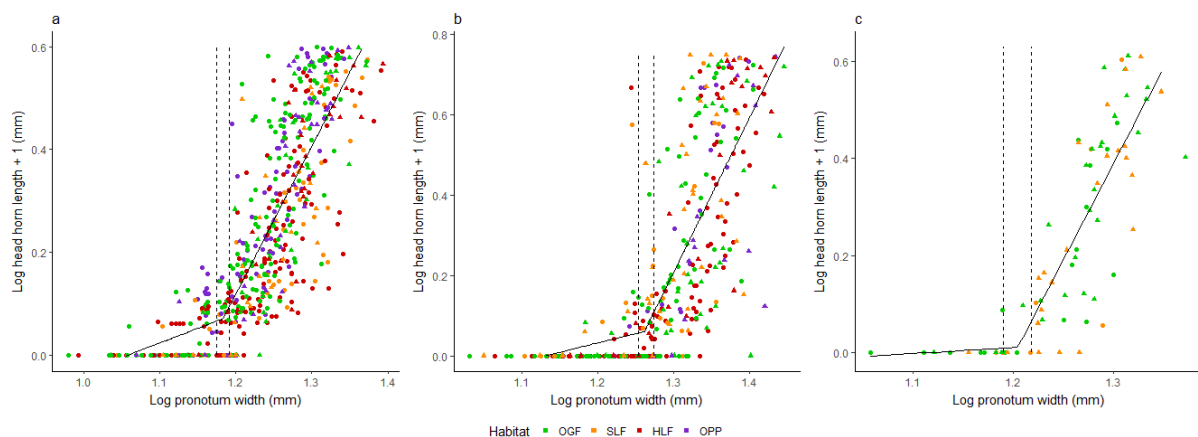


Figure 5.18: Allometric relationships between horn length and body size of (a) *O. obscurior*, (b) *O. cervicapra* and (c) *O. negroboscuro*, following the removal of asymptotic majors the switchpoint body size between minor and majors was determined using *segmented* (Muggeo, 2003) and showing standard errors (dashed lines) of (a) *O. obscurior*, (b) *O. cervicapra* and (c) *O. negroboscuro*. Habitat is indicated by colour (OGF: green; SLF: orange; HLF: red; and OPP: purple) and year indicated by point shape (2011: circle; 2015: triangle).

Table 5.2: Summary of AIC scores from GAMs fitted to horn length-body size allometry of *O. obscurior*, *O. cervicapra* and *O. negroboscuro* testing for differences in allometry between habitats and years.

Species	Model	Model variables	df	AIC
<i>O. obscurior</i>				
	GAM-1	Habitat + year + smoother (body size * habitat)	15	-684.87
	GAM-2	Habitat + smoother (body size * habitat)	14	-690.40
	GAM-3	Habitat + smoother (body size)	8	-731.46
	GAM-4	smoother (body size)	5	-743.57
<i>O. cervicapra</i>				
	GAM-1	Habitat + year + smoother (body size * habitat)	15	127.47
	GAM-2	Habitat + smoother (body size * habitat)	14	121.43
	GAM-3	Habitat + smoother (body size)	8	92.81
	GAM-4	smoother (body size)	5	78.62
<i>O. negroboscuro</i>				
	GAM-1	Habitat + year + smoother (body size * habitat)	9	-24.80
	GAM-2	Habitat + smoother (body size * habitat)	8	-31.35
	GAM-3	Habitat + smoother (body size)	6	-43.57
	GAM-4	smoother (body size)	5	-48.68

The proportion of male morphs found in each habitat only differed in *O. cervicapra*, indicated by a main effect of habitat ($\chi^2 = 14.68$, $df = 1$, $p = 0.002$; Figure 15.19b). A greater proportion of *O. cervicapra* males found within OPP were classified as majors compared to all other habitat types. There was no difference in the proportion of each morph found between habitats in *O. obscurior* ($\chi^2 = 0.05$, $df = 1$, $p = 0.997$) or *O. negroboscuro* ($\chi^2 = 0.03$, $df = 1$, $p = 0.956$). In all three species there was a significant main effect of year, all indicating that in 2015 a greater proportion of males were majors compared to 2011 (*O. obscurior*: $\chi^2 = 8.03$, $df = 1$, $p = 0.005$; *O. cervicapra*: $\chi^2 = 16.44$, $df = 1$, $p < 0.001$; *O. negroboscuro*: $\chi^2 = 8.26$, $df = 1$, $p = 0.004$; Figure 5.19)

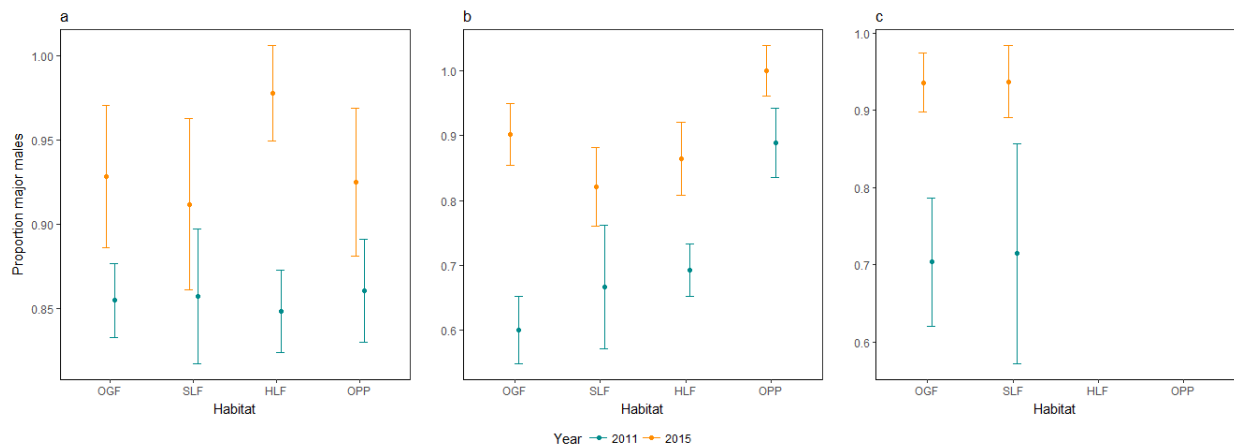


Figure 5.19: Proportion of males classified as major males in each habitat type (OGF = old growth forest, SLF = selectively logged forest, HLF = heavily logged forest and OPP = oil palm plantation) of (a) *O. obscurior*, (b) *O. cervicapra* and (c) *O. negroobscurior*. Green indicates beetles collected in 2011 and orange those collected in 2015.

There was no difference in the relative testes mass of *O. negroobscurior* between habitats ($\chi^2 = 4.69$, $df = 1$, $p = 0.196$), the minimal adequate model did however retain main effects of total mass ($\chi^2 = 25.09$, $df = 1$, $p < 0.001$) and horn length ($\chi^2 = 6.04$, $df = 1$, $p = 0.014$). Inspection of figure 5.20 and model coefficients indicates a positive relationship between total mass and testes mass, whereas a negative relationship exists between horn length and testes mass. None of the variation in relative testes mass of *O. obscurior* or *O. cervicapra* males was explained by any of the explanatory variable used in the models except total mass (*O. obscurior*: $\chi^2 = 40.37$, $df = 1$, $p < 0.001$; *O. cervicapra*: $\chi^2 = 64.49$, $df = 1$, $p < 0.001$).

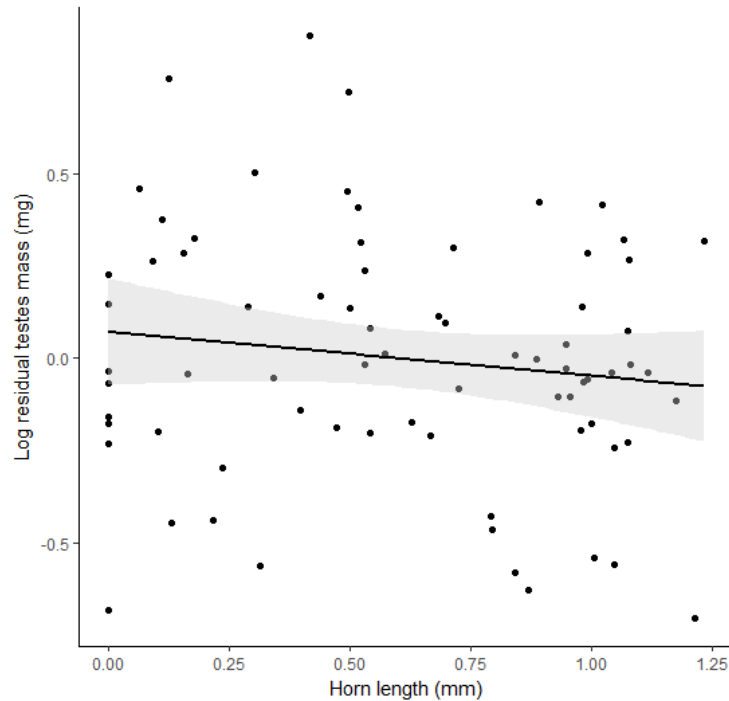


Figure 5.20: Testes mass of *O. negroboscuro* after the removal of the effects of body size and total mass against horn length.

5.4.2.4 Summary of the expression of sexually selected traits

Overall, the expression of horns is largely in accordance with how well a species is adapted to a habitat type. When species are found in habitats at high abundance and condition, individuals have relatively large horns and a greater proportion of males are classified as major males (i.e. *C. dayacus* and *P. watanabei*; Table 5.3). In contrast, when species are well adapted to most habitats across the disturbance gradient, the allometric relationship between horn length and body is not affected and the proportion of each male morph largely unaltered (i.e. *Onthophagus spp.*; Table 5.3). In all species there was no effect of habitat on the testes mass of individuals (Table 5.3).

Table 5.3: Summary table of results from horn length-body size allometry, proportion male morph and testes mass across the disturbance gradient in the six species investigated. Habitats listed in bold indicated the habitat/s species are likely to be most well adapted to (see table 5.1). Trait responses in bold indicate habitats with highest expression of trait.

<i>C. dayacus</i>			
Habitat	Horn- body allometry	Proportion major male	Testes mass
OGF	Relatively large horns	Highest proportion	No difference
SLF	Relatively large horns	Lowest proportion	No difference
HLF	Relatively small horns	Lowest proportion	No difference
OPP	-	-	-
<i>C. renaudpauliani</i>			
Habitat	Horn- body allometry	Proportion major male	Testes mass
OGF	-	-	-
SLF	No difference	Lowest proportion	No difference
HLF	No difference	Highest proportion	No difference
OPP	No difference	Highest proportion	No difference
<i>P. watanabei</i>			
Habitat	Horn- body allometry	Proportion major male	Testes mass
OGF	Relatively small horns – γ ♂	Lowest proportion	No difference
SLF	Relatively large horns	Reduced proportion	No difference
HLF	Relatively large horns	Highest proportion	No difference
OPP	Relatively large horns	Reduced proportion	No difference
<i>O. obscurior</i>			
Habitat	Horn- body allometry	Proportion major male	Testes mass
OGF	No difference	No difference	No difference
SLF	No difference	No difference	No difference
HLF	No difference	No difference	No difference
OPP	No difference	No difference	No difference
<i>O. cervicapra</i>			
Habitat	Horn- body allometry	Proportion major male	Testes mass
OGF	No difference	No difference	No difference
SLF	No difference	No difference	No difference
HLF	No difference	No difference	No difference
OPP	No difference	No difference	No difference
<i>O. negroboscuroi</i>			
Habitat	Horn- body allometry	Proportion major male	Testes mass
OGF	No difference	No difference	No difference
SLF	No difference	No difference	No difference
HLF	-	-	-
OPP	-	-	-

5.5 Discussion

Here I investigated the expression of sexually selected traits in altered habitats and using species abundance and individual condition as an estimate of how well adapted each species was within the respective habitat types. Largely in line with predictions, a general pattern emerges that when abundance is high within a habitat individuals are also in good condition. There are however a number of exceptions to this general pattern. Sexually selected traits were expected to differ between habitats in which beetles were close to or further away from their adaptive peaks, horn expression data is largely in agreement with these predictions. When a species is moved away from their adaptive peaks by environmental change this alters the expression of weaponry, reducing relative horn length and the proportion of major males, the latter may also alter the selective pressures experienced by each male morph. The supports the assumption that the expression of horns generally show patterns of condition dependence. In contrast, although within species condition tends to influence testes mass this apparent condition dependence does not appear to influence the expression of testes mass between habitats and against what was predicted. This difference is likely caused by how the expression of each trait is determined and differences in resources availability at different life stages.

5.5.1 *Catharsius* spp.

Comparing both *Catharsius* species abundance suggests that *C. dayacus* is an undisturbed forest specialist, but can survive in moderately disturbed habitats. In contrast *C. renaudpauliani* is a disturbed forest specialist, which can survive moving into OPP. The reason for this difference in distribution between *Catharsius* species is unknown and may reflect differences in dietary preferences and the changing mammalian communities in the different habitats (Wearn *et al.*, 2016). Alternatively, a possibly more likely explanation

could be related to thermal tolerances, with *C. renaudpauliani* being adapted to higher temperatures than *C. dayacus* (see Hardwick et al., 2015 for temperature differences between sites). Carrying out thermal tolerance experiments would be informative. In the habitats where both species survive i.e. SLF and HLF it is possible that the reduced abundances when moved away from optimal habitats reflects competition between the two species for resources, where *C. dayacus* out competes *C. renaudpauliani* in SLF and vice versa in HLF, possibly indicating that a carrying capacity exists within each habitat for species which fill similar niches.

Overall the body mass data from *C. dayacus* supports this view: when beetles are found in high abundance they are in good condition, whereas in HLF where overall abundance is low males are found to be in poor condition. Although a significant difference in condition of *C. renaudpauliani* was not detected between habitats, the data show a similar pattern of reduced condition in SLF reflecting the overall reduction in abundance. The reason for possibly not detecting a significant effect is likely a consequence of the low number of *C. renaudpauliani* trapped in SLF.

The expression of horns in *C. dayacus* corroborates this interpretation of condition differing within altered habitats. When moved from their adaptive peaks within HLF there is a reduction in relative horn length. This strongly suggests condition dependence of horn expression in this species. Furthermore, in both disturbed sites SLF and HLF a smaller proportion of males classified as majors, therefore in OGF more males express disproportionately larger horns. Again a similar non-significant pattern was found in *C. renaudpauliani* which suggests the same effect may be occurring in SLF when *C. renaudpauliani* males are moved from their environmental optima.

5.5.2 *Proagoderus watanabei*

The patterns of abundance and condition of *P. watanabei* strongly suggest that this species is most adapted to disturbed habitats (HLF), but we should note that *P. watanabei* also has a broad ecological niche and can persist in all habitats sampled from within this study. When comparing allometric relationships between habitat types there is generally no difference in the expression of horns, apart from in gamma males in OGF. Taken together these results suggest that generally horn expression is largely unaffected by habitat modification despite populations showing reduced abundances, suggesting populations suffer reduced productivity when moved from optimal habitats. There is an exception to this in OGF, presumably the habitat this species is least adapted to, whereby individuals are in the poorest condition and have a reduction in relative horn lengths in the smallest males. A greater proportion of males were classified as alpha males within HLF, again suggesting that this is the habitat *P. watanabei* is most suited to. The overall decrease in abundance as well as the observation of reduced proportion of alpha males moving away from HLF suggests that horn length is condition dependent. The scaling relationship between horn length and body size generally does not differ between habitats, a possible explanation for these results is that both brood ball size or quantity were reduced in OGF, SLF and OPP, but that if larvae are provided with ample resource they will still develop into alphas.

Previous examination of the scaling relation between both horn types (head and pronotum) and body size in *P. watanabei* have been carried out (Moczek, Brühl and Krell, 2004), however, the overall interpretation is different to what has been described here. Previously, head horn length-body size allometry has been described as a linear relationship and pronotum horn length-body size allometry as sigmoidal (Moczek, Brühl and Krell, 2004). Within the current study this is not the best way to describe these scaling relationships, although clear similarities exist, for example in the threshold-dependent development of

pronotum horns found in both studies. By incorporating this binary expression of the pronotum horn into the visualisation of head horn length-body size allometry and performing goodness of fit tests on models, these current data are best described as having three male morphs. The previously published study of allometric relationships in this species (Moczek, Brühl and Krell, 2004) did not incorporate both measurements in this way. It is important to note that visual inspection of figure 3 in Moczek et al., (2004) does show strong similarities with the interpretation provided here and it is possible that the increased sample size in the current study (304 compared to 70) made it possible to detect these different male morphs. Additionally, different methods were used to make measurements and it is possible that due to the shape of horns slightly different actual measurements were being made, however, the horn lengths described here and in Moczek et al., (2004) show extremely similar ranges, suggesting approximately the same measurements were being made. In both cases collections were carried out across a range of habitats and it is also possible that these different populations do actually exhibit different allometric relationships. It would be interesting to combine and compare data to test this.

5.5.3 *Onthophagus* spp.

Two of the three *Onthophagus* in the current study were found in all habitat types. In both cases there was a marked reduction in abundance in SLF. Why fewer *O. obscurior* and *O. cervicapra* were found in SLF but similar abundance in HLF to OGF is difficult to explain. One possible explanation could be due to dung freshness. The SLF regions sampled in this study have limited access therefore the bait balls were slightly older than those used in other habitat types. These species could be more attracted to fresh dung and use this strategy to avoid competition with larger species; if so, this may explain this pattern. Due to the high abundances in HLF these species are interpreted as being adapted to all forest habitats with *O. cervicapra* apparently only showing reduced abundance in OPP. The slight reduction in

condition of *O. obscurior* in SLF may go against this interpretation, however, individuals could be attracted to dung for two reasons, feeding and reproduction. An explanation for this could also be related to preferences for fresh dung, if *O. obscurior* is generally only attracted to fresh dung for reproduction, but will feed from older dung piles when necessary, it would be expected that individuals in poor condition would be attracted to older dung piles. Little or nothing is known about dung preferences in these species studied here, although dung preferences are known from other dung beetles (Whipple and Hoback, 2012).

Overall, there is little or no evidence that the *Onthophagus* species examined here show different expression of horns between habitats. This general lack of difference mirrors the finding that condition of beetles between habitats tends to not differ, again providing evidence that these species are likely to be reasonably well adapted to all habitats across the disturbance gradient (OGF and SLF only in *O. negroobscurior*). There was however, a significant difference in the proportion of major males in the populations between years in all three species. This possibly reflects yearly differences in resources available for developing larvae or that a change in abiotic factors had caused populations to be in poorer condition in 2011. Determining the cause for this is difficult as these effects were not also seen in the other species examined here. Although effort was made to have a random representation of individuals it is possible that an unforeseen bias towards capturing major males was occurring in these small species and this could be related to the smallest males escaping from the live traps used in 2015.

5.5.4 General patterns

Overall, these results corroborate the findings from a previous field study in which the expression of sexually dimorphic traits were reduced in newts, *Lissotriton helveticus*, which were found in agriculture plantations compared to native forest (Iglesias-Carrasco *et al.*,

2016). However, no overall population fitness estimates were made and how these changes in trait expression related to population fitness was not investigated. By incorporating abundance data in the current study this has allowed a better understanding of trait expression within the altered habitats, which without the overall interpretation would be very different due to species likely being better adapted to disturbed and undisturbed habitats. This highlights the importance of including abundance data in this type of study.

Furthermore, the condition data is largely in accordance with the abundance data and indicates that how well adapted species are to each habitat is reflected in both estimates of individual and population fitness. A notable exception to this is in *O. cervicapra* where in OPP there is reduced abundance but individual condition is high. Species specific dung preferences and variation in resources may explain this observation. In the three species with largest body sizes (*C. dayacus*, *C. renaudpauliani* and *P. watanabei*) there is a strong effect of male morph on mass, with major and alpha males tending to be in better condition. In another dung beetle, *Euoniticellus intermedius*, weight gain in males was associated with horn length and therefore indirectly with larval conditions (Reaney and Knell, 2015). The increased mass of majors in these three species may then be related to a similar mechanism; however this may be species specific or possibly related to overall body size. Furthermore, in *C. dayacus* this difference between morphs disappears in HLF suggesting that habitat disturbance could potentially alter a species' ability to assimilate food.

Within the species investigated here there was generally a strong effect of total mass after being controlled for body size on testes mass, indicating that males in good condition (high relative total mass) tend to have increased testes mass. These results strongly suggest that relative testes mass is condition dependent. Despite this and contrary to predictions we find no effect of habitat on relative testes mass in any species. This may be caused by differences in the relative strength of pre- and post-copulatory sexual selection in dung beetles (Almbro

and Simmons, 2014) or because of the proximate causes for the expression of horns and testes in holometabolous insects (Reaney and Knell, 2015). Horn lengths are determined during pupation and are dependent on larval resources which are largely set by brood ball size (Emlen, 1997b). In contrast, testes mass is likely to be variable and sensitive to adult nutrition (Knell and Simmons, 2010; Reaney and Knell, 2015). When male *O. taurus* were given poor and good quality diets no difference in testes mass was found even though the poor diet reduced beetle weight, and testes mass was only reduced when beetles were exposed to complete starvation (Knell and Simmons, 2010). In the current study, males are likely to still be feeding, but possibly with reduced quality or quantity, in all habitats which they are found and therefore despite having relatively poor condition can maintain the expression of their testes; this is a possible explanation for why testes mass did not differ between habitats in the current study.

Due to the asymmetry in strength of sperm competition each male morph is expected to face due to different reproductive strategies (Emlen, 1997), it has previously been shown that minor males invest more resources in and have relatively large testes compared major males (Simmons, Tomkins and Hunt, 1999; Tomkins and Simmons, 2000; Simmons, Emlen and Tomkins, 2007). This may also lead to condition dependence of testes in minors males, whereas major males testes are largely unaffected by condition (Knell and Simmons, 2010). It was predicted that similar effects would be observed in the current study. However, only weak support for differing investment in testes size between male morphs and being influenced by relative horn length was found. It is possible that males being starved for differing lengths of time before dissections were performed increased the noise in theses data making expected patterns masked and hard to detect.

Dung beetles are holometabolous insects with expression of horns determined during prepupal stages of the life cycle and largely based upon larval conditions and the resources

provide within the brood ball (Emlen, 1997b, 2000). In all species in the current study there is good evidence that male populations are polymorphic. Evidence from another polymorphic species, *O. taurus*, suggests that horn expression and suppression are based upon hormonal control towards the end of larval life stages (Emlen & Nijhout, 1999, 2001). Data presented here suggest that when populations are not within their optimal habitat, estimated from population abundance and condition then horn expression is reduced, but if species maintain similar abundances then horn expression is largely unaffected by habitat change. Since horn expression is mainly determined during larval stages, there are a number of possible explanations for this. Due to changing mammalian diversity and abundance (Wearn *et al.*, 2016) resources used to build brood balls are reduced, there may be increased competition from other species over resources, or changing abiotic factors (Hardwick *et al.*, 2015) may move species from their adaptive peaks. By performing translocation experiments it may be possible to disentangle the causes for trait expression being altered (Iglesias-Carrasco *et al.*, 2016).

Differences in horn expression are related to the reproductive strategies males adopt, with those large major males attempting to monopolise females and small minor males gaining sneak copulations (Emlen, 1997a). These data suggest that when populations are moved from their adaptive peaks the average male reproductive strategy in a population is also altered.

Habitat change has previously been shown to alter the relative reproductive success of different male reproductive strategies in bulb mites, *R. echinopus* (Tomkins *et al.*, 2011), and differing populations densities may also alter the relative reproductive success of each male morph and benefits of horn expression (Moczek *et al.*, 2002; Pomfret and Knell, 2008).

These data suggests that when populations are moved from their optimal habitat, a greater proportion of males will adopt minor male reproductive strategies within the population. This is likely to lead to increased sperm competition within a population and possibly a reduction

in the ability of high quality major males to monopolise females, potentially altering the effects sexual selection may have on adaptation to these altered habitats.

Overall, these data support the assumption of condition dependence of sexually selected traits. However, habitat change moving populations off their adaptive peaks influences the expression of pre- and post-copulatory sexually selected traits in different ways. Horn expression appears to only be affected in species which are moved from their optimal habitat type, whereas in species with wider ecological niches horn expression seems largely unaffected by changes in habitats. In contrast, there is no habitat effect on the expression of testes, despite there being good evidence for condition effecting expression. This is likely due to differences in how the expression of horns and testes is determined. If habitat change alters the expression of sexually selected traits then the mechanisms of sexual selection and mating system may also be altered, this may change the relative reproductive of success of males in good and poor condition and ultimately influence selection patterns and the effect sexual selection may have population fitness facing environmental change.

Chapter 6: General discussion

6.1 Thesis overview

In recent years, our understanding of the fundamental mechanisms of sexual selection have improved greatly (Rowe and Houle, 1996), and with these advances researchers have begun to question the role sexual selection may have on overall population fitness (Whitlock, 2000; Lorch *et al.*, 2003). In this thesis, I have examined the effect of sexual selection on population fitness when exposed to altered, changing and stable environments, investigating whether sexual selection enhances adaptation or increases extinction risk. Furthermore, I addressed how environmental change alters the expression of sexually selected traits and contrast this to population fitness, thus providing insight into which aspects of sexual selection are potentially driving the effects of sexual selection on population fitness. I approached these questions in two different ways; through experimental evolution and through a comparative field study. In general, previous research with regards to whether sexual selection influences adaptation rate and extinction risk find contrasting results between laboratory and field studies, with the former tending to show beneficial effects of sexual selection and the latter negative or no effect of sexual selection (e.g. Sorci, Møller and Clobert, 1998; Morrow and Fricke, 2004; Plesnar-Bielak *et al.*, 2012; Bro-Jørgensen, 2014; Lumley *et al.*, 2015).

Despite the ubiquity of sexual selection and the clear importance of understanding what aspects of a species evolution and ecology influence responses to environmental change there is a general lack of empirical investigation and a wider range of species are needed to assess general trends. This lack of diversity in groups of animals researched is particularly apparent with regards to field studies, with the vast majority of examples coming from vertebrates (e.g. birds: Sorci, Møller and Clobert, 1998; and mammals: Morrow & Fricke, 2004). Although there are far more laboratory studies, a large proportion of these set out to also examine

sexual conflict and model species selected because of their known interlocus sexual conflict , for example *Drosophila* (e.g. Rundle, Chenoweth and Blows, 2006). This may be skewing current perspectives on the wider implications of sexual selection on adaptation. Using methods and study systems which have not previously been investigated with regards to these questions, the work within this thesis represents an important empirical contribution to our understanding of the role of sexual selection on adaptation rates and population persistence.

Environmental change is widespread, but the rate and degree of this change is likely to differ between regions and ecosystems (Collins *et al.*, 2013). The rate and degree of environmental change could possibly alter the costs and benefits of sexual selection (Martínez-Ruiz and Knell, 2016). To date however, no research to my knowledge has empirically tested the effect of sexual selection on adaptation within a changing environment. This is potentially worrying as in most cases environmental change within ecosystems is likely to be gradual, and not the abrupt step changes which previous experimental work has used (e.g. Fricke & Arnqvist, 2007; Holland, 2002; Plesnar-Bielak *et al.*, 2012). I investigate this using an experimental evolution approach in which the strength of sexual selection was altered within populations and populations exposed to stable and gradually increasing temperatures to simulate climate change. Additionally, in most other studies when assessing fitness there is no manipulation of the opportunity for sexual selection to act on individuals. In this study by using a factorial design, individuals evolving with strong and weak sexual selection were exposed to a mating system treatment where sexual selection was allowed or removed by allowing polyandry or enforcing monogamy, respectively. This was done to gain a better understanding of evolved and phenotypic effects of sexual selection on individual fitness.

There are only a few field studies addressing the effect of sexual selection on extinction risk. A number of studies investigate this by using a large number of species and quantify the effect of sexual selection on population fitness by conservation status of species in question

(e.g. Bro-Jørgensen, 2014; Morrow & Fricke, 2004). Although clearly important these studies lack information regarding details to whether this is related to environmental change, or for example increased population turnover rates (Doherty *et al.*, 2003). Furthermore, because overall population sizes were not known issues of density dependent effects of expressing sexually selected traits may confound interpretation (Kokko and Rankin, 2006; Knell, 2009b). A notable exception comes from studies on birds historically introduced to islands (McLain, Moulton and Redfearn, 1995; Sorci, Møller and Clobert, 1998; McLain, Moulton and Sanderson, 1999), however, introduction of small population sizes could alter demographic processes and the effect sexual selection has on adaptation (Martínez-Ruiz and Knell, 2016).

I investigated the effect of sexually selected traits on species richness and persistence of species after the increased intensification of land use and modification of old-growth tropical rainforest to oil palm plantation. This study is unique in a number of ways. Addressing this question using insects has not been done in the field before. Actual abundance data was determined within each habitat type giving much greater detail than possible with previous studies. Furthermore, the abundance of populations investigated would have been at a natural level before modification and therefore issues of introduction effort and small population size confounding previous studies can largely be excluded. Finally, the habitats represent a gradient of disturbance which has rarely been possible to examine.

Furthermore, previous work has shown that different aspects of sexual selection vary in their relative strengths (Almbro and Simmons, 2014) and that environmental change can alter the expression of sexually selected traits (Iglesias-Carrasco *et al.*, 2016). By estimating the relative strength and condition dependence of different aspects of sexual selection and sexually selected traits (i.e. pre- and post-copulatory), I also investigate how sexually selected traits are influenced by environmental change and gain insight into which aspect of sexual

selection may be driving adaptive evolution or species persistence. This has rarely been done within the same study investigating the impacts of sexual selection on adaptation, meaning that previous research cannot highlight which aspect of sexual selection is driving the observed effects. Furthermore, due to the nature of field work, previous work has not been able to estimate both population fitness and the expression of sexually selected traits in altered habitats. By using insects as study systems I was able to have relatively large sample sizes and determine if variation in sexually selected traits exists between habitats and if this was related to overall population fitness.

6.2 Data chapter summaries

Chapter 2: This chapter used experimental evolution to examine the effect of sexual selection on population fitness when exposed to both increasing and stable temperatures. By manipulating adult sex ratios of populations of the Indian meal moth, *Plodia interpunctella*, the strength of sexual selection was altered to be either strong or weak, having male biased or female biased sex ratios, respectively. An extra treatment was included during fitness assays which allowed a comparison between both evolved and phenotypic effects of sexual selection; this was done by manipulating the mating system and enforcing monogamy or allowing polyandry. Most notably the fitness assays measured the lifetime fecundity of females and proportion offspring survival.

In the increasing temperature experiment there was a clear effect of increasing temperatures driving populations to extinction. Before extinction however, those populations with strong sexual selection showed increased offspring survival compared to populations with weak sexual selection. Moreover, there was a strong positive effect of polyandry on both female fecundity and offspring survival, indicating that females having choice over mating partners and mating with multiple males can significantly increase population fitness and this is

independent of evolutionary history. These results suggest that sexual selection can increase population fitness and the rate of adaptation to constantly changing temperatures, potentially providing a buffer against climate change.

In comparison, when populations were reared in stable temperatures these positive effects of sexual selection on population fitness largely disappeared. This indicates that under stabilising selection the benefits of sexual selection may be negated by sexual conflict.

Intralocus sexual conflict is known to be strong in *P. interpunctella* and this may explain why there was not an increase in population fitness. If strong sexual selection increases selection on males, sexually antagonistic evolution may pull females away from their optimal phenotype and decrease overall population fitness.

Chapter 3: By using the same experimental populations used in chapter 2 the strength of pre- and post-copulatory sexual selection was estimated. The attractiveness of males was quantified by performing mating assays, in which male success in gaining a mating with a standardised female was measured and how long the latent period to mating was. To gain an estimate of post-copulatory sexual selection testes dissection were performed. Under increasing temperatures there was no overall effect of the strength of sexual selection populations were evolving with on male mating success, this result however is possibly an effect of mating assays having to be performed at ancestral temperatures. There was however, no difference in mating latency of males suggesting that these differences in rearing and mating assay temperatures did not affect behaviour. It is probable that increased temperatures caused males to be in poorer condition overall. In contrast, under stable temperatures male mating success was significantly higher in those populations with strong sexual selection compared to weak sexual selection. Indicating that female choice in male biased populations was increasing the average attractiveness of males; this however was not associated with an increase in population fitness (chapter 2). In both experiments there was not a strong pattern

of testes size differing between sexual selection treatments or by increasing temperature.

There was however large variation within and between generation. These results indicate that female choice in *P. interpunctella* probably causes stronger selection on males than sperm competition.

Chapter 4: A field study was undertaken within a large scale ecological experiment in Sabah, Malaysian Borneo investigating the impacts of tropical rainforest modification and the conversion of rainforest to oil palm plantation. Dung beetles were sampled across the full gradient of habitat disturbance found at the SAFE project (Ewers *et al.*, 2011), with habitats ranging from old-growth rainforest to oil palm plantation. The strength of sexual selection each dung beetle species experienced was estimated from measuring sexually selected traits. A molecular phylogeny of the paracoprid nesters was made and used to control for phylogenetic relationships between species.

There was evidence that species richness and the abundance of species found in old-growth forest was reduced as habitat modification increased. This reduction in the persistence of species was however less in horned species. Furthermore, those species with relatively large horns also tended to persist across the disturbance gradient at higher abundances than species with relatively small horns. This suggests that species which engage in male-male competition and in which the intensity of this competition was high have an increased ability to adapt to and persist within altered environments. Additionally, the scaling relationship between horn length and body size was found to influence the abundance of species and suggests that different mechanisms of control over horn expression may have different costs in altered environments. There was a weak negative effect of testes mass on species persistence; however this relationship appears questionable. No effect of sexual size dimorphism on species persistence was found. These results suggest different sexually selected traits have trait specific costs and benefits. Overall though male-male competition

and the ability of males within a population to exclude rivals from gaining copulations has the strongest effect and probably acts to increase the spread of adaptive alleles throughout the population.

Chapter 5: In this chapter I selected six species of dung beetle to investigate how habitat modification affects the expression of sexually selected traits within a species and contrast this to estimates of how well adapted species are within these altered habitats. The dung beetles chosen to address this question were chosen due to them having ecological importance. Species abundance and individuals' condition was taken as an estimate of how well adapted each species was within each habitat. Overall these two measures were largely in agreement: when a species is found at high relative abundance within a habitat the individuals tended to be in good condition. By comparing horn length-body size allometry and the proportion of males which develop into majors between habitats, a pattern emerges that when species are moved from the habitat they are adapted to, horn expression is reduced. In those species which are found widely across all habitats this reduction in horn expression is not observed. These results suggest that horn expression is likely to be condition dependent and condition can be affected in certain species by habitat change. There was good evidence that testes mass is also condition dependent in these species studied. Testes mass was however, not affected by habitat change. This difference in the relationship of horn and testes expression between habitats is likely due to how the expression of each trait is determined and possibly a consequence of horn structures being fixed and testes mass being plastic.

6.3 Discussion

In this thesis, I have investigated the effect of sexual selection on species adaptation and persistence to environmental change. Furthermore, the impacts of environmental change on the expression of sexually selected traits were examined. In both study systems these results

largely show that sexual selection is beneficial to population survival when facing novel and changing environments. These results indicate that sexual selection is acting to skew reproductive success in favour of males with high genetic quality, thus enhancing the spread of beneficial adaptive alleles throughout the population. Additionally, identifying that it is probably pre-copulatory processes rather than post-copulatory which are driving these beneficial effects. This is in line with a number of theoretical investigations (Lorch *et al.*, 2003; Martínez-Ruiz and Knell, 2016).

The findings from chapters 2 and 3 using experimental evolution are largely in agreement with and corroborate the results from previous empirical investigation (Fricke and Arnqvist, 2007; Long, Agrawal and Rowe, 2012; Plesnar-Bielak *et al.*, 2012). Overall, I find that under directional selection there are positive effects of sexual selection, both evolved and phenotypic, to population fitness. In contrast, under stabilising selection these positive effects largely disappear. It was suggested by Long *et al.* (2012) that when variation in reproductive success is based on adaptive and non-adaptive alleles that the benefits of sexual selection should outweigh the costs of sexual conflict, but under stabilising selection variation in fitness will likely be caused by sexual conflict. When populations reside close to their adaptive peaks the benefits of sexual selection may be small. In contrast, when populations are further away from their adaptive peaks the benefits of sexual selection favouring adaptive beneficial alleles are likely to be increased. The mating success of males from populations with strong sexual selection increased within stable temperatures, but no such pattern exists under increasing temperatures and provides support for this hypothesis. Interestingly, in previous investigations species were known to have interlocus sexual conflict where males directly harm their mates (Fricke and Arnqvist, 2007; Long, Agrawal and Rowe, 2012), whereas, in *P. interpunctella* there is no evidence of direct harm to females from males. These results therefore suggest that intralocus sexual conflict could be an important factor in

the population fitness of *P. interpunctella* found within stable environments. This is in line with previous findings that intralocus sexual conflict is strong within *P. interpunctella* (Lewis et al., 2011). Overall, these results provide important empirical evidence to improve our understanding of a broad evolutionary ecology question that has widespread implications within our changing world. Additionally, furthering our understanding of the reproductive biology and adaptive potential of a *P. interpunctella* may have implications to problems related to food wastage caused by this widespread pest species of stored food products (Mohandass et al., 2007).

In comparison, although my findings from chapter 4 are largely in line with theory of sexual selection increasing adaptation rates and species niche width (Proulx, 1999, 2002; Lorch et al., 2003; Martínez-Ruiz and Knell, 2016), they are in conflict with previous comparative field studies. All of the previous comparative field research show negative effects of sexual selection on population persistence within novel environments (McLain, Moulton and Redfearn, 1995; Sorci, Møller and Clobert, 1998; McLain, Moulton and Sanderson, 1999). This difference may be related to overall population sizes in the current study being larger than those in contrasting studies (e.g. Sorci, Møller and Clobert, 1998). Population size is potentially important in determining the relationship between sexual selection and adaptation, and my findings suggest that the negative effects of sexual selection caused by demographic stochasticity are possibly minimized when populations are large and corroborate suggestions by Martínez-Ruiz and Knell (2016). I find evidence that horn and testes expression in those dung beetles species investigated are likely to be condition dependent in chapter 5 and this is line with previous work (Emlen, 1997b; Knell & Simmons, 2010). The observation that only expression of horns, and not testes, were altered when species are moved from their optimal habitat is likely a consequence of how expression of traits is determined. Taken together with findings that the expression of horns and relative horn length is positively associated with

population persistence in altered habitats suggests that male-male contests over matings are under stronger selection than sperm competition, similar suggestions have been made from the dung beetle, *Onthophagus taurus* (Almbro and Simmons, 2014). Dung beetles are an ecologically important group which provide a number of ecosystem services, including seed dispersal and dung removal (Slade *et al.*, 2007; Nichols *et al.*, 2008), therefore the impacts of habitat modification on their diversity and abundance could have wider implications other than loss of biodiversity. More generally, this research provides evidence that species with strong sexual selection, most notably male-male contest competition, may be less prone to extinction when habitats are modified and this could be important information when planning for conservation and biodiversity management.

6.4 Future directions

6.4.1 *Plodia interpunctella*

It is evident that sexual selection can enhance adaptation rates to increasing temperature in *P. interpunctella*. Whether this could also be replicated with a change in a different environmental factor would be a clear avenue for future work. Additionally, it would be interesting to investigate changes in multiple environmental factors, for example including a diet treatment; however the work load clearly would be substantial. Male mating success appears to be under strong selection, but little is known about this within *P. interpunctella*. I was unable to detect any difference in male mating success when populations were experiencing increasing temperatures. Due to the observed increase in fitness however, this strongly suggests that variance in male reproductive success exists. Future work could examine this variation by quantifying lifetime number of matings or ability to seduce non-virgin females and whether either of these are associated with the observed increase in adaptation rates. Additionally, investigating what signals in male ‘song’ and courtship display

are used by females to judge male quality and whether these show strong condition dependence could provide useful insights. The polyandrous mating system treatment was shown to increase female fitness under increasing temperatures, providing both direct and indirect benefits. Whether females were mating with specific males or mating multiply was not determined in the current study, but with a clear effect this may also be a consideration for future work to explore.

Sperm competition in *P. interpunctella* has previously been shown to be important in reproduction within this species, particularly size and contents of ejaculate. Here though no meaningful patterns in the expression of testes were found, it would be interesting to investigate this further. Males produce two distinct types of sperm, non-fertile and fertile, and both types are believed to be important in sperm competition. It has previously been assumed that non-fertile sperm is cheaper to produce than fertile sperm, whether this relaxes selection on testes size could be an interesting study. Through a comparative study, relative testes size and proportion of sperm types could be compared and it may be possible to address this.

6.4.2 Dung beetles

Overall, clear patterns of condition dependence of both horn and testes expression were found within species. This led to different expression of horn lengths between habitat types and differences in the proportion of each male morph in a number of species; how this affected male reproductive success is unknown. Future work could address how different proportions of each male morph, at different population densities effects sexual selection under directional selection and how the relative reproductive success of each morph is changed. Clearly, a laboratory approach probably with *Onthophagus taurus* as the model system would suit this question.

Differences in the allometric relationship of horn length and body size were shown to influence species abundance across the disturbance gradient. Results here suggest discontinuous allometries are less adaptive within an altered environment, possibly due to the more probabilistic nature of horn expression in discontinuous allometries which may lead to increased mismatch between phenotype and environment. Future work could investigate the differences in the mechanisms of horn expression between those species in which horn expression is determined by a threshold body size and those with more probabilistic horn expression over a wide range of body sizes.

Further investigation into *Catharsius dayacus* and *Catharsius renaudpauliani* is warranted. It is currently not known what causes these species to be adapted to undisturbed and disturbed forest habitats. Whether the reduced survival of species is caused by temperature tolerances, dung preference or due to competition with the other species would be interesting to address. Initially, translocation experiments using mesocosms could prove useful.

Within this thesis the effect of pre- and post-copulatory sexual selection was determined and the possible effects each aspect of sexual selection may have on adaptation. Although both assumed to occur little is known about the relative strengths of female choice and male-male competition and the effect of each could have on population fitness. This relates to the relative amount of reproductive skew caused by each mechanism of sexual selection, and whether the outcome of each mechanism is effective at favouring males of high genetic quality. An experiment to test this could be carried out in a dung beetle, but another model system with both weaponry and courtship display known to be condition dependent could equally be used.

6.5 Final conclusions

In conclusion, by using empirical techniques I have investigated the role of sexual selection in adaptation and persistence within altered and changing habitats. Due to the increased amount of reproductive skew in males caused by variation in the expression of sexually selected traits, which is likely to be based upon individuals' condition. I have shown that sexual selection can favour those males with high genetic quality, most suited to the altered environment, thus spreading beneficial adaptive alleles throughout the population. This may have wide implications with regards to biodiversity and conservation management, helping to address which species may be more vulnerable to or able to persist within altered and changing environments. However, also highlighting that the effects of sexual selection are varied and more research is needed if any general patterns are to be found.

Appendices

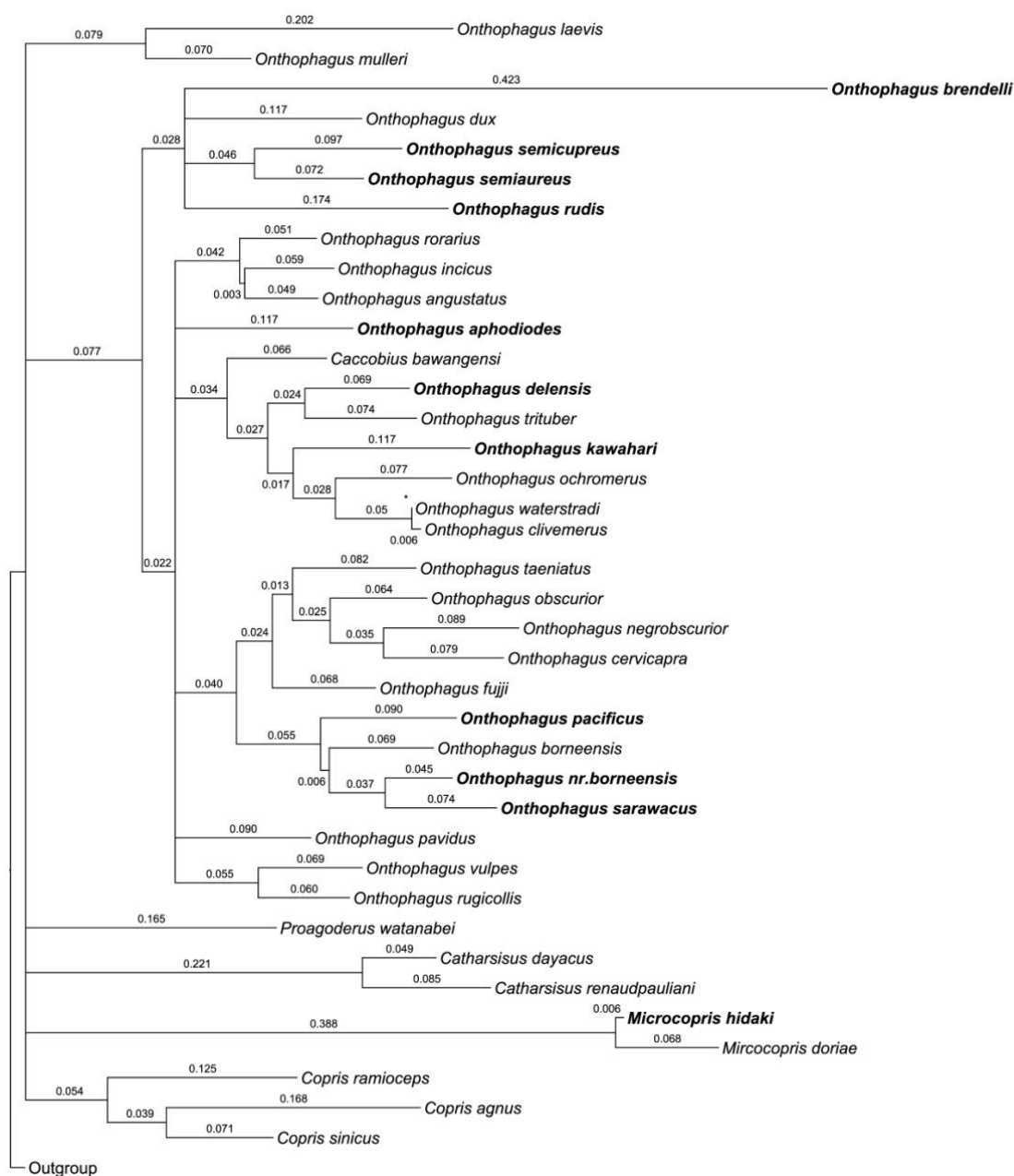


Figure 4.3A: Full tunneler molecular phylogram. Phylogram made using TreeGraph 2 (Stöver and Müller, 2010) of maximum likelihood tree of the *cox1* gene the 36 dung beetle species and rooted using sequences of three *Aphodius* species as an outgroup. Hornless species are in bold. Nodes with <50% bootstrap values have been collapsed. Numbers indicate genetic distance of branches with the branch labelled with an asterisk having a value of 6.2×10^{-6} .

References

- Agrawal, A. F. (2001) 'Sexual selection and the maintenance of sexual reproduction.', *Nature*, 411(6838), pp. 692–695. doi: 10.1038/35079590.
- Ala-Honkola, O., Laine, L., Pekkala, N., Kotiaho, J. S., Honkola, T. and Puurtinen, M. (2015) 'Males Benefit from Mating with Outbred Females in *Drosophila littoralis*: Male Choice for Female Genetic Quality?', *Ethology*, 121(6), pp. 577–585. doi: 10.1111/eth.12369.
- Alix, B., Boubacar, D. A. and Vladimir, M. (2012) 'T-REX: A web server for inferring, validating and visualizing phylogenetic trees and networks', *Nucleic Acids Research*, 40(W1), pp. 573–579. doi: 10.1093/nar/gks485.
- Allen, B. J. and Levinton, J. S. (2007) 'Costs of bearing a sexually selected ornamental weapon in a fiddler crab', *Functional Ecology*, 21(1), pp. 154–161. doi: 10.1111/j.1365-2435.2006.01219.x.
- Almbro, M. and Simmons, L. W. (2014) 'Sexual selection can remove an experimentally induced mutation load', *Evolution*, 68(1), pp. 295–300. doi: 10.1111/evo.12238.
- Andersson, M. (1994) *Sexual Selection*. New Jersey: Princeton University Press.
- Andersson, M. and Simmons, L. W. (2006) 'Sexual selection and mate choice', *Trends in Ecology and Evolution*, 21(6), pp. 296–302. doi: 10.1016/j.tree.2006.03.015.
- Arbuthnott, D. and Rundle, H. D. (2012) 'Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*', *Evolution*, 66(7), pp. 2127–2137. doi: 10.1111/j.1558-5646.2012.01584.x.
- Arnqvist, G. and Nilsson, T. (2000) 'The evolution of polyandry: multiple mating and female fitness in insects.', *Animal behaviour*, 60(2), pp. 145–164. doi: 10.1006/anbe.2000.1446.
- Arnqvist, G. and Rowe, L. (1995) 'Sexual conflict and arms races between the sexes: a morphological adaptation for control of mating in a female insect', *Proceedings of the Royal Society B: Biological Sciences*, 261, pp. 123–127.
- Arnqvist, G. and Tuda, M. (2010) 'Sexual conflict and the gender load: correlated evolution between population fitness and sexual dimorphism in seed beetles', *Proceedings of the Royal Society B*, 277(December 2009), pp. 1345–1352. doi: 10.1098/rspb.2009.2026.
- Ashcroft, M. B. and Gollan, J. R. (2012) 'Fine-resolution (25 m) topoclimatic grids of near-surface (5 cm) extreme temperatures and humidities across various habitats in a large (200 ?? 300 km) and diverse region', *International Journal of Climatology*, 32(14), pp. 2134–2148. doi: 10.1002/joc.2428.
- Bangham, J., Chapman, T. and Partridge, L. (2002) 'Effects of body size, accessory gland and testis size on pre-and postcopulatory success in *Drosophila melanogaster*', *Animal Behaviour*, 64, pp. 915–921. doi: 10.1006.
- Bateman, A. J. (1948) 'Intra-sexual selection in *Drosophila*', *Heredity*, 2(3), pp. 349–368. doi: 10.1038/hdy.1948.21.
- Bates, D., Mächler, M., Bolker, B. M. and Walker, S. C. (2015) 'Fitting linear mixed-effects

models using lme4', *Journal of Statistical Software*, 67(1–48), pp. 1–51. doi: 10.18637/jss.v067.i01.

Bernasconi, G. and Keller, L. (2001) 'Female polyandry affects their sons' reproductive success in the four beetle *Tribolium castaneum*', *Journal of Evolutionary Biology*, 14, pp. 186–193. doi: 10.1046/j.1420-9101.2001.00247.x.

Bertin, A. and Cézilly, F. (2003) 'Sexual selection, antennae length and the mating advantage of large males in *Asellus aquaticus*', *Journal of Evolutionary Biology*, 16(3), pp. 491–500. doi: 10.1046/j.1420-9101.2003.00536.x.

Birkhead, T. R. (1998) 'Cryptic Female Choice : Criteria for Establishing Female Sperm Choice', *Evolution*, 52(4), pp. 1212–1218. doi: 10.2307/2411251.

Blomberg, S. P., Garland, T. and Ives, A. R. (2003) 'Testing for phylogenetic signal in comparative data: behavioral traits are more labile', *Evolution*, 57(4), pp. 717–745. doi: 10.1111/j.0014-3820.2003.tb00285.x.

Bocedi, G. and Reid, J. M. (2015) 'Evolution of female multiple mating: A quantitative model of the "sexually selected sperm" hypothesis', *Evolution*, 69(1), pp. 39–58. doi: 10.1111/evo.12550.

Bonduriansky, R. (2001) 'The evolution of male mate choice in insects: a synthesis of ideas and evidence', *Biological Reviews*, 76(3), pp. 305–339. doi: 10.1017/s1464793101005693.

Bonduriansky, R. and Chenoweth, S. F. (2009) 'Intralocus sexual conflict', *Trends in Ecology & Evolution*, pp. 280–288. doi: 10.1016/j.tree.2008.12.005.

Boorman, E. and Parker, G. A. (1976) 'Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status', *Ecological Entomology*, 1(3), pp. 145–155. doi: 10.1111/j.1365-2311.1976.tb01217.x.

Bradshaw, C. J. A., Sodhi, N. S. and Brook, B. W. (2009) 'Tropical turmoil: A biodiversity tragedy in progress', *Frontiers in Ecology and the Environment*, 7(2), pp. 79–87. doi: 10.1890/070193.

Brady, U. E., Tumlinson, J. H., Brownlee, R. G. and Silverstein, R. M. (1971) 'Sex stimulant and attractant in the Indian meal moth and in the almond moth.', *Science (New York, N.Y.)*, 171(3973), pp. 802–804. doi: 10.1126/science.171.3973.802.

Braga, R. F., Korasaki, V., Andresen, E. and Louzada, J. (2013) 'Dung Beetle Community and Functions along a Habitat-Disturbance Gradient in the Amazon: A Rapid Assessment of Ecological Functions Associated to Biodiversity', *PLoS ONE*, 8(2). doi: 10.1371/journal.pone.0057786.

Brandt, L. S. E. and Greenfield, M. D. (2004) 'Condition-dependent traits and the capture of genetic variance in male advertisement song', *Journal of Evolutionary Biology*, 17(4), pp. 821–828. doi: 10.1111/j.1420-9101.2004.00716.x.

Bro-Jørgensen, J. (2007) 'The intensity of sexual selection predicts weapon size in male bovids', *Evolution*, 61(6), pp. 1316–1326. doi: 10.1111/j.1558-5646.2007.00111.x.

Bro-Jørgensen, J. (2014) 'Will their armaments be their downfall? Large horn size increases

- extinction risk in bovids', *Animal Conservation*, 17(1), pp. 80–87. doi: 10.1111/acv.12062.
- Bro-Jørgensen, J. and Durant, S. M. (2003) 'Mating strategies of topi bulls: getting in the centre of attention', *Animal Behaviour*, 65, pp. 585–594. doi: 10.1006/anbe.2003.2077.
- Brockmann, H., Nguyen, C. and Potts, W. (2000) 'Paternity in horseshoe crabs when spawning in multiple-male groups.', *Animal behaviour*, 60(6), pp. 837–849. doi: 10.1006/anbe.2000.1547.
- Browne, J. and Scholtz, C. (1999) 'A phylogeny of the families of Scarabaeoidea (Coleoptera)', *Systematic Entomology*, 24(1), pp. 51–84. doi: 10.1046/j.1365-3113.1999.00067.x.
- Bulmer, M. G. and Parker, G. A. (2002) 'The evolution of anisogamy: a game-theoretic approach', *Proceedings of the Royal Society B: Biological Sciences*, 269(1507), pp. 2381–2388. doi: 10.1098/rspb.2002.2161.
- Burger, R. and Lynch, M. (1995) 'Evolution and Extinction in a Changing Environment - a Quantitative-Genetic Analysis', *Evolution*, 49(1), pp. 151–163. doi: 10.1029/2007GL032057.
- Burkhardt, D. and de la Motte, I. (1988) 'Big "antlers" are favoured: female choice in stalk-eyed flies (Diptera, Insecta), field collected harems and laboratory experiments', *Journal of Comparative Physiology A*, 162(5), pp. 649–652. doi: 10.1007/BF01342640.
- Byrne, P. G., Roberts, J. D. and Simmons, L. W. (2002) 'Sperm competition selects for increased testes mass in Australian frogs', *Journal of Evolutionary Biology*, 15(3), pp. 347–355. doi: 10.1046/j.1420-9101.2002.00409.x.
- Calbacho-Rosa, L., Córdoba-Aguilar, A. and Peretti, A. (2010) 'Occurrence and duration of post-copulatory mate guarding in a spider with last sperm precedence', *Behaviour*, 147(10), pp. 1267–1283. doi: 10.1163/000579510X514544.
- Callander, S., Kahn, A. T., Hunt, J., Backwell, P. R. Y. and Jennions, M. D. (2013) 'The effect of competitors on calling effort and life span in male field crickets', *Behavioral Ecology*, 24(5), pp. 1251–1259. doi: 10.1093/beheco/art059.
- Candolin, U. and Heuschele, J. (2008) 'Is sexual selection beneficial during adaptation to environmental change?', *Trends in Ecology and Evolution*, pp. 446–452. doi: 10.1016/j.tree.2008.04.008.
- Candolin, U., Salesto, T. and Evers, M. (2007) 'Changed environmental conditions weaken sexual selection in sticklebacks', *Journal of Evolutionary Biology*, 20(1), pp. 233–239. doi: 10.1111/j.1420-9101.2006.01207.x.
- Chapman, T. (2001) 'Seminal fluid-mediated fitness traits in *Drosophila*', *Heredity*, pp. 511–521. doi: 10.1046/j.1365-2540.2001.00961.x.
- Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L. (2003) 'Sexual conflict', *Trends in Ecology & Evolution*, 18(1), pp. 41–47. doi: 10.1016/S0169-5347(02)00004-6.
- Chapman, T. and Davies, S. J. (2004) 'Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies', *Peptides*, 25(9), pp. 1477–1490. doi: 10.1016/j.peptides.2003.10.023.

- Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. and Partridge, L. (1995) 'Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products.', *Nature*, pp. 241–244. doi: 10.1038/373241a0.
- Chapman, T., Neubaum, D. M., Wolfner, M. F. and Partridge, L. (2000) 'The role of male accessory gland protein Acp36DE in sperm competition in *Drosophila melanogaster*.', *Proceedings. Biological sciences / The Royal Society*, 267(1448), pp. 1097–1105. doi: 10.1098/rspb.2000.1114.
- Chenoweth, S. F., Appleton, N. C., Allen, S. L. and Rundle, H. D. (2015) 'Genomic Evidence that Sexual Selection Impedes Adaptation to a Novel Environment', *Current Biology*. Elsevier Ltd, 25(14), pp. 1860–1866. doi: 10.1016/j.cub.2015.05.034.
- Chevin, L. M., Lande, R. and Mace, G. M. (2010) 'Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory', *PLoS Biology*, 8(4), pp. 1–8. doi: 10.1371/journal.pbio.1000357.
- Chippindale, A. K., Gibson, J. R. and Rice, W. R. (2001) 'Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*', *Proceedings of the National Academy of Sciences of the United States of America*, 98(4), pp. 1671–1675. doi: 10.1073/pnas.98.4.1671.
- Christy, J. H. and Salmon, M. (1984) 'Ecology and evolution of mating systems of fiddler crabs (genus *Uca*)', *Biological Reviews*, 59, pp. 483–509.
- Clutton-Brock, T. H., Albon, S. D., Gibson, R. M. and Guinness, F. E. (1979) 'The Logical Stag: Adaptive Aspect of Fighting in Red Deer (*Cervus Elaphus* L.)', *Animal Behaviour*, 27, pp. 211–225.
- Clutton-Brock, T. H. and Langley, P. (1997) 'Persistent courtship reduces male and female longevity in captive tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae)', *Behavioral Ecology*, 8(4), pp. 392–395. doi: 10.1093/beheco/8.4.392.
- Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W. J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A. J. and Wehner, M. (2013) 'Long-term Climate Change: Projections, Commitments and Irreversibility', in Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M. (eds) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, pp. 1029–1136. doi: 10.1017/CBO9781107415324.024.
- Cook, J. M. and Bean, D. (2006) 'Cryptic male dimorphism and fighting in a fig wasp', *Animal Behaviour*, 71(5), pp. 1095–1101. doi: 10.1016/j.anbehav.2005.07.027.
- Cook, J. M., Bean, D. and Power, S. (1999) 'Fatal fighting in fig wasps - GBH in time and space', *Trends in Ecology and Evolution*, 14(99), pp. 257–259. doi: 10.1016/S0169-5347(99)01661-4.
- Cook, P. A. (1999) 'Sperm numbers and female fertility in the moth *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae).', *Journal of Insect Behavior*, 12(6), pp. 767–779. doi: 10.1023/a:1020952909933.

- Cook, P. A. and Gage, M. J. G. (1995) 'Effects of risk of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by moth *Plodia interpunctella* (Lepidoptera: Pyralidae)', *Behavioral Ecology and Sociobiology*, pp. 261–268. doi: 10.1007/s002650050148.
- Cook, P. A. and Wedell, N. (1999) 'Non-fertile sperm delay female remating', *Nature*, 397(6719), p. 486. doi: 10.1038/17257.
- Cotton, S., Fowler, K. and Pomiankowski, A. (2004a) 'Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)', *Evolution*, 58(5), p. 1038. doi: 10.1554/03-514.
- Cotton, S., Fowler, K. and Pomiankowski, A. (2004b) 'Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis?', *Proceedings. Biological sciences / The Royal Society*, 271(1541), pp. 771–783. doi: 10.1098/rspb.2004.2688.
- Cotton, S., Small, J. and Pomiankowski, A. (2006) 'Sexual Selection and Condition-Dependent Mate Preferences', *Current Biology*, pp. 755–765. doi: 10.1016/j.cub.2006.08.022.
- Crudginton, H. S. and Siva-Jothy, M. T. (2000) 'Genital damage, kicking and early death.', *Nature*, 407, pp. 855–856. doi: 10.1038/35038154.
- Dakin, R. and Montgomerie, R. (2014) 'Condition-dependent mate assessment and choice by peahens: Implications for sexual selection', *Behavioral Ecology*, 25(5), pp. 1097–1104. doi: 10.1093/beheco/aru087.
- Danielsson, I. (2001) 'Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (*Gerris lacustris*).', *Proceedings. Biological sciences / The Royal Society*, 268(1462), pp. 77–81. doi: 10.1098/rspb.2000.1332.
- Darwin, C. (1859) *The Origin of Species*,. Oxford: Oxford World's Classics.
- Darwin, C. (1871) *The Descent of Man and Selection in Relation to Sex*. London: Murray. doi: 10.1038/011305a0.
- David, P., Bjorksten, T., Fowler, K. and Pomiankowski, a (2000) 'Condition-dependent signalling of genetic variation in stalk-eyed flies.', *Nature*, 406(6792), pp. 186–188. doi: 10.1038/35018079.
- Davis, A. J., Holloway, J. D., Huijbregts, H., Krikken, J., Kirk-spriggs, A. H. and Sutton, S. L. (2001) 'Dung beetles as indicators af change in the forests of northern Borneo', *Journal of Applied Ecology*, 38, pp. 593–616.
- Demary, K. C. and Lewis, S. M. (2007) 'Male courtship attractiveness and paternity success in *Photinus greeni* fireflies', *Evolution*, 61(2), pp. 431–439. doi: 10.1111/j.1558-5646.2007.00033.x.
- Doherty, P. F., Sorci, G., Royle, J. A., Hines, J. E., Nichols, J. D. and Bouludier, T. (2003) 'Sexual selection affects local extinction and turnover in bird communities', *Proceedings of the National Academy of Sciences*, 100(10), pp. 5858–5862. doi: 10.1073/pnas.0836953100.
- Dolgin, E. S., Whitlock, M. C. and Agrawal, A. F. (2006) 'Male *Drosophila melanogaster*

- have higher mating success when adapted to their thermal environment', *Journal of Evolutionary Biology*, 19(6), pp. 1894–1900. doi: 10.1111/j.1420-9101.2006.01168.x.
- Van Doorn, G. S. (2009) 'Intralocus sexual conflict', *Annals of the New York Academy of Sciences*, pp. 52–71. doi: 10.1111/j.1749-6632.2009.04573.x.
- Drewsbury, D. A. (1982) 'Ejaculate cost and male choice', *The American Naturalist*, 119(5), pp. 601–610. doi: DOI: 10.1086/283938.
- Eberhard, W. G. (1996) *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.
- Edwards, F. A., Edwards, D. P., Larsen, T. H., Hsu, W. W., Benedick, S., Chung, A., Vun Khen, C., Wilcove, D. S. and Hamer, K. C. (2013) 'Does logging and forest conversion to oil palm agriculture alter functional diversity in a biodiversity hotspot?', *Animal Conservation*, 17(2), pp. 163–173. doi: 10.1111/acv.12074.
- Edwards, F. A., Finan, J., Graham, L. K., Larsen, T. H., Wilcove, D. S., Hsu, W. W., Chey, V. K. and Hamer, K. C. (2017) 'The impact of logging roads on dung beetle assemblages in a tropical rainforest reserve', *Biological Conservation*. The Authors, 205, pp. 85–92. doi: 10.1016/j.biocon.2016.11.011.
- Egan, A. L., Hook, K. A., Reeve, H. K. and Iyengar, V. K. (2016) 'Polyandrous females provide sons with more competitive sperm: Support for the sexy-sperm hypothesis in the rattlebox moth (*Utetheisa ornatrix*)', *Evolution*, 70(1), pp. 72–81. doi: 10.1111/evo.12829.
- Eggert, A. K. (1992) 'Alternative male mate-finding tactics in burying beetles', *Behavioral Ecology*, 3(3), pp. 243–254. doi: 10.1093/beheco/3.3.243.
- Elias, D. O., Sivalinghem, S., Mason, A. C., Andrade, M. C. B. and Kasumovic, M. M. (2014) 'Mate-guarding courtship behaviour: Tactics in a changing world', *Animal Behaviour*, 97, pp. 25–33. doi: 10.1016/j.anbehav.2014.08.007.
- Emlen, D. J. (1994) 'Environmental Control of Horn Length Dimorphism in the Beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae)', *Proceedings of the Royal Society B: Biological Sciences*, 256(1346), pp. 131–136. doi: 10.1098/rspb.1994.0060.
- Emlen, D. J. (1996) 'Artificial Selection on Horn Length-Body Size Allometry in the Horned Beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae)', *Evolution*, pp. 1219–1230. doi: 10.2307/2410662.
- Emlen, D. J. (1997a) 'Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae)', *Behavioral Ecology and Sociobiology*, 41(5), pp. 335–341. doi: 10.1007/s002650050393.
- Emlen, D. J. (1997b) 'Diet alters male horn allometry in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae)', *Proceedings of the Royal Society B: Biological Sciences*, 264(1381), pp. 567–574. doi: 10.1098/rspb.1997.0081.
- Emlen, D. J. (2000) 'Integrating Development with Evolution: A Case Study with Beetle Horns', *BioScience*, 50(5), p. 403. doi: 10.1641/0006-3568(2000)050[0403:IDWEAC]2.0.CO;2.
- Emlen, D. J. (2001) 'Costs and the diversification of exaggerated animal structures.', *Science*,

291(5508), pp. 1534–6. doi: 10.1126/science.291.5508.1534.

Emlen, D. J. (2008) ‘The Evolution of Animal Weapons’, *Annual Review of Ecology, Evolution, and Systematics*, 39(1), pp. 387–413. doi: 10.1146/annurev.ecolsys.39.110707.173502.

Emlen, D. J., Hunt, J. and Simmons, L. W. (2005) ‘Evolution of sexual dimorphism and male dimorphism in the expression of beetle horns: phylogenetic evidence for modularity, evolutionary lability, and constraint.’, *The American Naturalist*, 166, suppl(october), pp. S42–S68. doi: 10.1086/444599.

Emlen, D. J., Marangelo, J., Ball, B. and Cunningham, C. W. (2005) ‘Diversity in the Weapons of Sexual Selection: Horn Evolution in the Beetle Genus *Onthophagus* (Coleoptera: Scarabaeidae)’, *Evolution*, 59(5), pp. 1060–1084. doi: 10.1111/j.0014-3820.2005.tb01044.x.

Emlen, D. J. and Nijhout, H. F. (1999) ‘Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae)’, *Journal of Insect Physiology*, 45(1), pp. 45–53. doi: 10.1046/j.1420-9101.1999.00004.x.

Emlen, D. J. and Nijhout, H. F. (2000) ‘The development and evolution of exaggerated morphologies in insects’, *Annual Review of Entomology*, 45, pp. 661–708. doi: 10.1146/annurev.ecolsys.36.102003.152640.

Emlen, D. J. and Nijhout, H. F. (2001) ‘Hormonal control of male horn length dimorphism in *Onthophagus taurus* (Coleoptera: Scarabaeidae): A second critical period of sensitivity to juvenile hormone’, *Journal of Insect Physiology*, 47(9), pp. 1045–1054. doi: 10.1016/S0022-1910(01)00084-1.

Emlen, S. and Oring, L. (1977) ‘Ecology, sexual selection, and the evolution of mating systems’, *Science*, pp. 215–223. doi: 10.1126/science.327542.

Evans, J. P., Zane, L., Francescato, S. and Pilastro, A. (2003) ‘Directional postcopulatory sexual selection revealed by artificial insemination’, *Nature*, 421(6921), pp. 360–363. doi: 10.1038/nature01367.

Evans, S. R. and Gustafsson, L. (2017) ‘Climate change upends selection on ornamentation in a wild bird’, *Nature Ecology & Evolution*, 1(2), p. 39. doi: 10.1038/s41559-016-0039.

Ewers, R. M., Didham, R. K., Fahrig, L., Ferraz, G., Hector, a., Holt, R. D., Kapos, V., Reynolds, G., Sinun, W., Snaddon, J. L. and Turner, E. C. (2011) ‘A large-scale forest fragmentation experiment: the Stability of Altered Forest Ecosystems Project’, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1582), pp. 3292–3302. doi: 10.1098/rstb.2011.0049.

Fedorka, K. M. and Mousseau, T. A. (2002) ‘Material and genetic benefits of female multiple mating and polyandry’, *Animal Behaviour*, 64(3), pp. 361–367. doi: 10.1006/anbe.2002.3052.

Firman, R. C., Gasparini, C., Manier, M. K. and Pizzari, T. (2017) ‘Postmating Female Control : 20 Years of Cryptic Female Choice’, *Trends in Ecology & Evolution*. Elsevier Ltd, 32(5), pp. 368–382. doi: 10.1016/j.tree.2017.02.010.

Fisher, R. A. (1930) *The genetical theory of natural selection*. Oxford: Clarendon press.

Flynn, D. F. B., Gogol-Prokurat, M., Nogeire, T., Molinari, N., Richers, B. T., Lin, B. B.,

- Simpson, N., Mayfield, M. M. and DeClerck, F. (2009) 'Loss of functional diversity under land use intensification across multiple taxa', *Ecology Letters*, 12(1), pp. 22–33. doi: 10.1111/j.1461-0248.2008.01255.x.
- Foerster, K., Coulson, T., Sheldon, B. C., Pemberton, J. M., Clutton-Brock, T. H. and Kruuk, L. E. B. (2007) 'Sexually antagonistic genetic variation for fitness in red deer.', *Nature*, 447(7148), pp. 1107–10. doi: 10.1038/nature05912.
- Forsgren, E., Kvarnemo, C. and Lindstrom, K. (1996) 'Mode of Sexual Selection Determined by Resource Abundance in Two Sand Goby Populations', *Evolution*, 50(2), pp. 646–654. doi: 10.2307/2410838.
- Fricke, C. and Arnqvist, G. (2007) 'Rapid adaptation to a novel host in a seed beetle (*Callosobruchus maculatus*): The role of sexual selection', *Evolution*, 61(2), pp. 440–454. doi: 10.1111/j.1558-5646.2007.00038.x.
- Friedländer, M. (1997) 'Control of the Eupyrene – Apyrene Sperm Dimorphism in Lepidoptera', *Journal of Insect Physiology*, 43(12), pp. 1085–1092.
- Gage, M. J. G. (1998) 'Influences of sex, size, and symmetry on ejaculate expenditure in a moth', *Behavioral Ecology*, 9(6), pp. 592–597. doi: 10.1093/beheco/9.6.592.
- Gage, M. J. G. and Cook, P. A. (1994) 'Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidae)', *Functional Ecology*, 8(5), pp. 594–599. doi: 10.2307/2389920.
- García-González, F. and Simmons, L. W. (2011) 'Good genes and sexual selection in dung beetles (*Onthophagus taurus*): Genetic variance in egg-to-adult and adult viability', *PLoS ONE*, 6(1), p. e16233. doi: 10.1371/journal.pone.0016233.
- Gasparini, C. and Pilastro, A. (2011) 'Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy', *Proceedings of the Royal Society B: Biological Sciences*, 278(1717), pp. 2495–2501. doi: 10.1098/rspb.2010.2369.
- Ghaderi, D., Springer, S. a, Ma, F., Cohen, M., Secrest, P., Taylor, R. E., Varki, A. and Gagneux, P. (2011) 'Sexual selection by female immunity against paternal antigens can fix loss of function alleles.', *Proceedings of the National Academy of Sciences of the United States of America*, 108(43), pp. 17743–8. doi: 10.1073/pnas.1102302108.
- Gienapp, P. and Merilä, J. (2010) 'Genetic and environmental effects on a condition-dependent trait: Feather growth in Siberian jays', *Journal of Evolutionary Biology*, 23(4), pp. 715–723. doi: 10.1111/j.1420-9101.2010.01949.x.
- Gilpin, M. E. and Soulé, M. E. (1986) 'Minimum viable populations: processes of extinction', in Soulé, M. E. (ed.) *Conservation biology: the science of scarcity and diversity*. Sunderland: MA: Sinauer Associates, pp. 19–34.
- Godin, J. G. J. and McDonough, H. E. (2003) 'Predator preference for brightly colored males in the guppy: A viability cost for a sexually selected trait', *Behavioral Ecology*, 14(2), pp. 194–200. doi: 10.1093/beheco/14.2.194.
- Gray, C. L., Slade, E. M., Mann, D. J. and Lewis, O. T. (2014) 'Do riparian reserves support dung beetle biodiversity and ecosystem services in oil palm-dominated tropical landscapes?', *Ecology and Evolution*, 4(7), pp. 1049–1060. doi: 10.1002/ece3.1003.

- Gray, D. A. and Eckhardt, G. (2001) 'Is cricket courtship song condition dependent?', *Animal Behaviour*, 62(5), pp. 871–877. doi: 10.1006/anbe.2001.1825.
- Gwynne, D. T. and Simmons, L. W. (1990) 'Experimental reversal of courtship roles in an insect', *Nature*, 346(1), pp. 172–174. doi: 10.1038/346172a0.
- Hall, M., Lindholm, A. K. and Brooks, R. (2004) 'Direct selection on male attractiveness and female preference fails to produce a response.', *BMC evolutionary biology*, 4, p. 1. doi: 10.1186/1471-2148-4-1.
- Hanks, L. M., Millar, J. G. and Paine, T. D. (1996) 'Body size influences mating success of the Eucalyptus longhorned borer (Coleoptera: Cerambycidae)', *Journal of Insect Behavior*, 9(3), pp. 369–382. doi: 10.1007/BF02214016.
- Hardwick, S. R., Toumi, R., Pfeifer, M., Turner, E. C., Nilus, R. and Ewers, R. M. (2015) 'The relationship between leaf area index and microclimate in tropical forest and oil palm plantation: Forest disturbance drives changes in microclimate', *Agricultural and Forest Meteorology*. Elsevier B.V., 201, pp. 187–195. doi: 10.1016/j.agrformet.2014.11.010.
- Harrison, S. J., Thomson, I. R., Grant, C. M. and Bertram, S. M. (2013) 'Calling, Courtship, and Condition in the Fall Field Cricket, *Gryllus pennsylvanicus*', *PLoS ONE*, 8(3). doi: 10.1371/journal.pone.0060356.
- Harrison, X. A. (2014) 'Using observation-level random effects to model overdispersion in count data in ecology and evolution.', *PeerJ*, 2, p. e616. doi: 10.7717/peerj.616.
- Harvey, P. H. and Pagel, M. D. (1991) *The comparative method in evolutionary biology*. Oxford: Oxford University Press. doi: 10.1016/0169-5347(92)90117-T.
- Heuschele, J., Salminen, T. and Candolin, U. (2012) 'Habitat change influences mate search behaviour in three-spined sticklebacks', *Animal Behaviour*. Elsevier Ltd, 83(6), pp. 1505–1510. doi: 10.1016/j.anbehav.2012.03.027.
- Hoffmann, A. A. and Sgro, C. M. (2011) 'Climate change and evolutionary adaptation', *Nature*, 470(7335), pp. 479–485. doi: 10.1038/nature09670.
- Hoffmann, A. A., Sgrò, C. M. and Kristensen, T. N. (2017) 'Revisiting Adaptive Potential, Population Size, and Conservation', *Trends in Ecology & Evolution*. Elsevier Ltd, 32(7), pp. 506–517. doi: 10.1016/j.tree.2017.03.012.
- Holland, B. (2002) 'Sexual selection fails to promote adaptation to a new environment.', *Evolution*, 56(4), pp. 721–30. doi: 10.1111/j.0014-3820.2002.tb01383.x.
- Holland, B. and Rice, W. R. (1999) 'Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load.', *Proceedings of the National Academy of Sciences of the United States of America*, 96(9), pp. 5083–5088. doi: 10.1073/pnas.96.9.5083.
- Hollis, B., Fierst, J. L. and Houle, D. (2009) 'Sexual selection accelerates the elimination of a deleterious mutant in *Drosophila melanogaster*', *Evolution*, 63(2), pp. 324–333. doi: 10.1111/j.1558-5646.2008.00551.x.
- Holman, L. and Kokko, H. (2013) 'The consequences of polyandry for population viability, extinction risk and conservation', *Philosophical Transactions of the Royal Society B*:

Biological Sciences, 368(1613), p. 20120053. doi: 10.1098/rstb.2012.0053.

Hosken, D. J. (1997) 'Sperm competition in bats.', *Proceedings. Biological sciences / The Royal Society*, 264(1380), pp. 385–392. doi: 10.1098/rspb.1997.0055.

Hosken, D. J. (2003) 'Sperm biology: Size indeed matters', *Current Biology*, 13(9), pp. 355–356. doi: 10.1016/S0960-9822(03)00275-6.

Hosken, D. J., Taylor, M. L., Hoyle, K., Higgins, S. and Wedell, N. (2008) 'Attractive males have greater success in sperm competition', *Current Biology*, 18(13), pp. 553–554. doi: 10.1016/j.cub.2008.04.028.

Hosken, D. J. and Ward, P. I. (2001) 'Experimental evidence for testes size evolution via sperm competition', *Ecology Letters*, 4, pp. 10–13.

House, C. M. and Simmons, L. W. (2006) 'Offensive and defensive sperm competition roles in the dung beetle *Onthophagus taurus* (Coleoptera : Scarabaeidae)', *Behavioral Ecology and Sociobiology*, 60(2), pp. 131–136. doi: 10.1007/s00265-005-0149-x.

Houston, A. I., Székely, T. and McNamara, J. M. (2005) 'Conflict between parents over care', *Trends in Ecology and Evolution*, 20(1), pp. 33–38. doi: 10.1016/j.tree.2004.10.008.

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and Williams, S. E. (2012) 'Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation.', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 367(1596), pp. 1665–79. doi: 10.1098/rstb.2012.0005.

Hunt, J., Breuker, C. J., Sadowski, J. A. and Moore, A. J. (2009) 'Male-male competition, female mate choice and their interaction: Determining total sexual selection', *Journal of Evolutionary Biology*, 22(1), pp. 13–26. doi: 10.1111/j.1420-9101.2008.01633.x.

Hunt, J., Brooks, R. and Jennions, M. D. (2005) 'Female mate choice as a condition-dependent life-history trait.', *The American naturalist*, 166(1), pp. 79–92. doi: 10.1086/430672.

Hunt, J. and Simmons, L. W. (1998) 'Patterns of parental provisioning covary with male morphology in a horned beetle (*Onthophagus taurus*) (Coleoptera: Scarabaeidae)', *Behavioral Ecology and Sociobiology*, 42(6), pp. 447–451. doi: 10.1007/s002650050459.

Hunt, J. and Simmons, L. W. (2001) 'Status-dependent selection in the dimorphic beetle *Onthophagus taurus*.', *Proceedings. Biological sciences / The Royal Society*, 268(1484), pp. 2409–2414. doi: 10.1098/rspb.2001.1758.

Hurtado-Gonzales, J. L. and Uy, J. A. C. (2010) 'Intrasexual competition facilitates the evolution of alternative mating strategies in a colour polymorphic fish.', *BMC evolutionary biology*, 10, p. 391. doi: 10.1186/1471-2148-10-391.

Iglesias-Carrasco, M., Head, M. L., Jennions, M. D. and Cabido, C. (2016) 'Condition-dependent trade-offs between sexual traits, body condition and immunity: the effect of novel habitats', *BMC Evolutionary Biology*. *BMC Evolutionary Biology*, 16(1), p. 135. doi: 10.1186/s12862-016-0706-0.

Ingleby, F. C., Lewis, Z. and Wedell, N. (2010) 'Level of sperm competition promotes evolution of male ejaculate allocation patterns in a moth', *Animal Behaviour*. Elsevier Ltd,

80(1), pp. 37–43. doi: 10.1016/j.anbehav.2010.03.022.

Ives, A. R. and Helmus, M. R. (2011) ‘Generalized linear mixed models for phylogenetic analyses of community structure’, *Ecological Monographs*, 81(3), pp. 511–525. doi: 10.1890/10-1264.1.

Jacomb, F., Marsh, J. and Holman, L. (2016) ‘Sexual selection expedites the evolution of pesticide resistance’, *Evolution*, 70(12), pp. 2746–2751. doi: 10.1111/evo.13074.

Jang, Y. and Greenfield, M. D. (1996) ‘Ultrasonic communication and sexual selection in wax moths: female choice based on energy and asynchrony of male signals’, *Animal Behaviour*, 51(September 1997), pp. 1095–1106. doi: 10.1006/anbe.1996.0111.

Jang, Y. and Greenfield, M. D. (1998) ‘Absolute Versus Relative Measurements of Sexual Selection: Assessing the Contributions of Ultrasonic Signal Characters to Mate Attraction in Lesser Wax Moths, *Achroia grisella* (Lepidoptera: Pyralidae)’, *Evolution*, 52(5), pp. 1383–1393. doi: 10.2307/2411308.

Jarošík, V. and Honek, A. (2007) ‘Sexual differences in insect development in relation to sexual size dimorphism’, in Fairbairn, D. J., Blanckenhorn, W. U., and Székely, T. (eds) *Sex, size and gender roles*. Oxford: Oxford University Press, pp. 205–212.

Järvenpää, M. and Lindström, K. (2004) ‘Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*.’, *Proceedings. Biological sciences / The Royal Society*, 271(1555), pp. 2361–2365. doi: 10.1098/rspb.2004.2870.

Jarzebowska, M. and Radwan, J. (2010) ‘Sexual selection counteracts extinction of small populations of the bulb mites’, *Evolution*, 64(5), pp. 1283–1289. doi: 10.1111/j.1558-5646.2009.00905.x.

Jennings, D. J. and Gammell, M. P. (2013) ‘Contest behaviour in ungulates’, in *Animal contests*. Cambridge: Cambridge University Press, pp. 304–320.

Jennions, M. D. and Petrie, M. (1997) ‘Variation in Mate Choice and Mating Preferences: a Review of Causes and Consequences’, *Biological Reviews*, 72(2), pp. 283–327. doi: 10.1111/j.1469-185X.1997.tb00015.x.

Jennions, M. D. and Petrie, M. (2000) ‘Why do females mate multiply? A review of the genetic benefits’, *Biological Reviews*, 75(1), pp. 21–64. doi: 10.1017/s0006323199005423.

Johnsen, A., Delhey, K., Andersson, S. and Kempenaers, B. (2003) ‘Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects’, *Proceedings of the Royal Society B: Biological Sciences*, 270(1521), pp. 1263–1270. doi: 10.1098/rspb.2003.2375.

Jormalainen, V. (1998) ‘Precopulatory Mate Guarding in Crustaceans: Male Competitive Strategy and Intersexual Conflict’, *The Quarterly Review of Biology*, pp. 275–304. doi: 10.2307/3036917.

Kemp, D. J. and Wiklund, C. (2001) ‘Fighting without weaponry: A review of male-male contest competition in butterflies’, *Behavioral Ecology and Sociobiology*, pp. 429–442. doi: 10.1007/s002650100318.

- Keyser, A. J. and Hill, G. E. (1999) 'Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament', *Proceedings of the Royal Society B: Biological Sciences*, 266(1421), pp. 771. doi: 10.1098/rspb.1999.0704.
- Kirkpatrick, M. and Ryan, M. J. (1991) 'The evolution of mating preferences and the paradox of the lek', *Nature*, 350(6313), pp. 33–38. doi: 10.1038/350033a0.
- Knell, R. J. (2009a) 'On the analysis of non-linear allometries', *Ecological Entomology*, 34(1), pp. 1–11. doi: 10.1111/j.1365-2311.2008.01022.x.
- Knell, R. J. (2009b) 'Population density and the evolution of male aggression', *Journal of Zoology*, 278(2), pp. 83–90. doi: 10.1111/j.1469-7998.2009.00566.x.
- Knell, R. J., Pomfret, J. C. and Tomkins, J. L. (2004) 'The limits of elaboration: curved allometries reveal the constraints on mandible size in stag beetles.', *Proceedings. Biological sciences / The Royal Society*, 271(March), pp. 523–528. doi: 10.1098/rspb.2003.2641.
- Knell, R. J. and Simmons, L. W. (2010) 'Mating tactics determine patterns of condition dependence in a dimorphic horned beetle.', *Proceedings. Biological sciences / The Royal Society*, 277(1692), pp. 2347–2353. doi: 10.1098/rspb.2010.0257.
- Knell, R. J. and Webberley, K. M. (2004) 'Sexually transmitted diseases of insects: distribution, evolution, ecology and host behaviour', *Biological Reviews*, 79, pp. 557–581. doi: 10.1017/S1464793103006365.
- Kokko, H. and Brooks, R. (2003) 'Sexy to die for? Sexual selection and the risk of extinction', *Annales Zoologici Fennici*, 40(April), pp. 207–219. doi: 10.2307/23736526.
- Kokko, H. and Rankin, D. J. (2006) 'Lonely hearts or sex in the city? Density-dependent effects in mating systems.', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 361(1466), pp. 319–334. doi: 10.1098/rstb.2005.1784.
- Kotiaho, J. S. (2002) 'Sexual selection and condition dependence of courtship display in three species of horned dung beetles', *Behavioral Ecology*, 13(6), pp. 791–799. doi: 10.1093/beheco/13.6.791.
- Kotiaho, J. S. and Simmons, L. W. (2003) 'Longevity cost of reproduction for males but no longevity cost of mating or courtship for females in the male-dimorphic dung beetle *Onthophagus binodis*', *Journal of Insect Physiology*, 49(9), pp. 817–822. doi: 10.1016/S0022-1910(03)00117-3.
- Kotiaho, J. S., Simmons, L. W. and Tomkins, J. L. (2001) 'Towards a resolution of the lek paradox', *Nature*, 410(6829), pp. 684–686. doi: 10.1038/35070557.
- Lailvaux, S. P., Reaney, L. T. and Backwell, P. R. Y. (2009) 'Dishonest signalling of fighting ability and multiple performance traits in the fiddler crab *Uca mjoebergi*', *Functional Ecology*, 23(2), pp. 359–366. doi: 10.1111/j.1365-2435.2008.01501.x.
- Lamanna, J. A. and Martin, T. E. (2016) 'Logging impacts on avian species richness and composition differ across latitudes and foraging and breeding habitat preferences', *Biological Reviews*, 92, pp. 1657–1674. doi: 10.1111/brv.12300.
- Larsen, T. H., Lopera, A. and Forsyth, A. (2006) 'Extreme trophic and habitat specialization by Peruvian dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae)', *Coleop. Bull.*, 60(5), pp.

315–324. doi: doi:10.1649/0010-065X(2006)60[315:ETAHSB]2.0.CO;2.

Larsen, T. H., Williams, N. M. and Kremen, C. (2005) 'Extinction order and altered community structure rapidly disrupt ecosystem functioning', *Ecology Letters*, 8(5), pp. 538–547. doi: 10.1111/j.1461-0248.2005.00749.x.

Lehtonen, J., Jennions, M. D. and Kokko, H. (2012) 'The many costs of sex', *Trends in Ecology and Evolution*. Elsevier Ltd, 27(3), pp. 172–178. doi: 10.1016/j.tree.2011.09.016.

Lewis, S. M. and Austad, S. N. (1994) 'Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness', *Behavioral Ecology*, 5(2), pp. 223–224. doi: 10.1093/beheco/5.2.223.

Lewis, Z. (2005) *Sexual selection and sexual conflict in the Lepidoptera*. University of Leeds.

Lewis, Z., Lizé, A. and Wedell, N. (2013) 'The interplay between different stages of reproduction in males of the moth *Plodia interpunctella*', *Animal Behaviour*. Elsevier Ltd, 86(5), pp. 917–922. doi: 10.1016/j.anbehav.2013.08.006.

Lewis, Z., Wedell, N. and Hunt, J. (2011) 'Evidence for strong intralocus sexual conflict in the indian meal moth, *plodia interpunctella*', *Evolution*, 65(7), pp. 2085–2097. doi: 10.1111/j.1558-5646.2011.01267.x.

Li, X. W., Fail, J. and Shelton, A. M. (2015) 'Female multiple matings and male harassment and their effects on fitness of arrhenotokous *Thrips tabaci* (Thysanoptera: Thripidae)', *Behavioral Ecology and Sociobiology*, 69(10), pp. 1585–1595. doi: 10.1007/s00265-015-1970-5.

Long, T. A. F., Agrawal, A. F. and Rowe, L. (2012) 'The effect of sexual selection on offspring fitness depends on the nature of genetic variation', *Current Biology*. Elsevier Ltd, 22(3), pp. 204–208. doi: 10.1016/j.cub.2011.12.020.

Lorch, P. D., Proulx, S., Rowe, L. and Day, T. (2003) 'Condition dependent sexual selection can accelerate adaptation.', *Evolution Ecology Research*, 5(6), pp. 867–881.

Lumley, A. J., Michalczyk, Ł., Kitson, J. J. N., Spurgin, L. G., Morrison, C. A., Godwin, J. L., Dickinson, M. E., Martin, O. Y., Emerson, B. C., Chapman, T. and Gage, M. J. G. (2015) 'Sexual selection protects against extinction', *Nature*, 522(7557), pp. 470–473. doi: 10.1038/nature14419.

Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R. and Brooks, R. C. (2008) 'Sex-Specific Fitness Effects of Nutrient Intake on Reproduction and Lifespan', *Current Biology*, 18(14), pp. 1062–1066. doi: 10.1016/j.cub.2008.06.059.

Mappes, J., Alatalo, R. V., Kotiaho, J. and Parri, S. (1996) 'Viability Costs of Condition-Dependent Sexual Male Display in a Drumming Wolf Spider', *Proceedings of the Royal Society B: Biological Sciences*, 263(1371), pp. 785–789. doi: 10.1098/rspb.1996.0117.

Markow, T. a (2000) 'Forced Matings in Natural Populations of *Drosophila*', *American Naturalist*, 156(1), pp. 155–156. doi: 10.1258/00236770781036508.

Marsh, C. J., Louzada, J., Beiroz, W. and Ewers, R. M. (2013) 'Optimising Bait for Pitfall Trapping of Amazonian Dung Beetles (Coleoptera: Scarabaeinae)', *PLoS ONE*, 8(8), pp. 1–8.

doi: 10.1371/journal.pone.0073147.

Martínez-Ruiz, C. and Knell, R. J. (2016) 'Sexual selection can both increase and decrease extinction probability: reconciling demographic and evolutionary factors', *Journal of Animal Ecology*, 86(1), pp. 117–127. doi: 10.1111/1365-2656.12601.

Mateos, C. and Carranza, J. (1996) 'On the intersexual selection for spurs in the ring-necked pheasant', *Behavioral Ecology*, 7(3), pp. 362–369. doi: 10.1093/beheco/7.3.362.

Matthews, I. M. and Magurran, A. E. (2000) 'Evidence for sperm transfer during sneaky mating in wild Trinidadian guppies', *Journal of Fish Biology*, 56(6), pp. 1381–1386. doi: 10.1006/jfbi.2000.1259.

Mccullough, E. L., Ledger, K. J., O'Brien, D. M. and Emlen, D. J. (2015) 'Variation in the allometry of exaggerated rhinoceros beetle horns', *Animal Behaviour*. Elsevier Ltd, 109, pp. 133–140. doi: 10.1016/j.anbehav.2015.08.013.

McGuigan, K., Van Homrigh, A. and Blows, M. W. (2008) 'An evolutionary limit to male mating success', *Evolution*, 62(6), pp. 1528–1537. doi: 10.1111/j.1558-5646.2008.00379.x.

McLachlan, A. J. and Allen, D. F. (1987) 'Male mating success in Diptera: advantages of small size', *Oikos*, 48(1), pp. 11–14.

McLain, D. K., Moulton, M. P. and Redfearn, T. P. (1995) 'Sexual selection and the risk of extinction of introduced birds on oceanic islands', *Oikos*, 74(1), pp. 27–34.

McLain, D. K., Moulton, M. P. and Sanderson, J. G. (1999) 'Sexual selection and extinction: The fate of plumage-dimorphic and plumage-monomorphic birds introduced onto islands', *Evolutionary Ecology Research*, 1, pp. 549–565.

McLain, D. K. and Vives, S. P. (1998) 'Sexual selection and community structure: An island biogeographic analysis with beetles', *Oikos*, 82(2), pp. 271–281. doi: 10.2307/3546967.

McNamara, K. B., Van Lieshout, E. and Simmons, L. W. (2014) 'A test of the sexy-sperm and good-sperm hypotheses for the evolution of polyandry', *Behavioral Ecology*, 25(4), pp. 989–995. doi: 10.1093/beheco/aru067.

Michalczyk, L., Millard, A. L., Martin, O. Y., Lumley, A. J., Emerson, B. C. and Gage, M. J. G. (2011) 'Experimental evolution exposes female and male responses to sexual selection and conflict in *tribolium castaneum*', *Evolution*, 65(3), pp. 713–724. doi: 10.1111/j.1558-5646.2010.01174.x.

Mineau, P., Derrickson, S. R. and McKinney, F. (1983) 'Forced Copulation in Waterfowl', *Behaviour*, pp. 250–293. doi: 10.1163/156853983X00390.

Moczek, A. P., Brühl, G. A. and Krell, F. T. (2004) 'Linear and threshold-dependent expression of secondary sexual traits in the same individual: Insights from a horned beetle (Coleoptera: Scarabaeidae)', *Biological Journal of the Linnean Society*, 83(4), pp. 473–480. doi: 10.1111/j.1095-8312.2004.00405.x.

Moczek, A. P. and Emlen, D. J. (2000) 'Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes?', *Animal behaviour*, 59(2), pp. 459–466. doi: 10.1006/anbe.1999.1342.

- Moczek, A. P., Hunt, J., Emlen, D. J. and Simmons, L. W. (2002) 'Threshold evolution in exotic populations of a polyphenic beetle', *Evolution Ecology Research*, 4(4), pp. 587–601. doi: 10.1046/j.1525-142X.2002.02014.x.
- Mohandass, S., Arthur, F. H., Zhu, K. Y. and Throne, J. E. (2007) 'Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products', *Journal of Stored Products Research*, 43(3), pp. 302–311. doi: 10.1016/j.jspr.2006.08.002.
- Møller, A. P. (1991) 'Sperm competition, sperm deletion, paternal care, and relative testis size in birds', *American Naturalist*, 137(6), pp. 882–906. doi: 10.1086/285199.
- Møller, A. P. and Jennions, M. D. (2001) 'How important are direct fitness benefits of sexual selection?', *Naturwissenschaften*, 88(10), pp. 401–415. doi: 10.1007/s001140100255.
- Monaghan, M. T., Inward, D. J. G., Hunt, T. and Vogler, A. P. (2007) 'A molecular phylogenetic analysis of the Scarabaeinae (dung beetles)', *Molecular Phylogenetics and Evolution*, 45(2), pp. 674–692. doi: 10.1016/j.ympev.2007.06.009.
- Morrow, E. H. and Fricke, C. (2004) 'Sexual selection and the risk of extinction in mammals', *Proceedings of the Royal Society of London B*, 271(1555), pp. 2395–2401. doi: 10.1098/rspb.2004.2888.
- Morrow, E. H. and Gage, M. J. G. (2000) 'The evolution of sperm length in moths.', *Proceedings. Biological sciences / The Royal Society*, 267, pp. 307–313. doi: 10.1098/rspb.2000.1001.
- Morrow, E. H. and Gage, M. J. G. (2001) 'Sperm competition experiments between lines of crickets producing different sperm lengths', *Proceedings of the Royal Society B: Biological Sciences*, 268(1482), pp. 2281–2286. doi: 10.1098/rspb.2001.1807.
- Morrow, E. H. and Pitcher, T. E. (2003) 'Sexual selection and the risk of extinction in birds', *Proceedings of the Royal Society of London B*, 270, pp. 1793–1799. doi: 10.1098/rspb.2004.2888.
- Muggeo, V. M. R. (2003) 'Estimating regression models with unknown break-points', *Statistics in Medicine*, 22(19), pp. 3055–3071. doi: 10.1002/sim.1545.
- Nakano, R., Takanashi, T., Fujii, T., Skals, N., Surlykke, a and Ishikawa, Y. (2009) 'Moths are not silent, but whisper ultrasonic courtship songs.', *The Journal of experimental biology*, 212(Pt 24), pp. 4072–4078. doi: 10.1242/jeb.032466.
- Nichols, E., Larsen, T. H., Spector, S., Davis, a L., Escobar, F., Favila, M. and Vulinec, K. (2007) 'Global dung beetle response to tropical forest modification and fragmentation: A quantitative literature review and meta-analysis', *Biological Conservation*, 137(1), pp. 1–19. doi: 10.1016/j.biocon.2007.01.023.
- Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezcuita, S. and Favila, M. E. (2008) 'Ecological functions and ecosystem services provided by Scarabaeinae dung beetles', *Biological Conservation*, 141(6), pp. 1461–1474. doi: 10.1016/j.biocon.2008.04.011.
- Nicoletto, P. F. (1993) 'Female sexual response to condition-dependent ornaments in the guppy, *Poecilia reticulata*', *Animal Behaviour*, pp. 441–450. doi: 10.1006/anbe.1993.1213.
- Nowicki, S., Searcy, W. A. ., Hughes, M. and Searcy, W. A. . (1998) 'The territory defense

- function of song in Song sparrows: A test with the speaker occupation design', *Behaviour*, 135(5), pp. 615–628. doi: 10.1163/156853998792897888.
- Ohlsson, T., Smith, H. G., Raberg, L. and Hasselquist, D. (2002) 'Pheasant sexual ornaments reflect nutritional conditions during early growth', *Proceedings of the Royal Society B: Biological Sciences*, 269(1486), pp. 21–27. doi: 10.1098/rspb.2001.1848.
- Ojanguren, A. and Magurran, A. E. (2007) 'Male harassment reduces short-term female fitness in guppies', *Behaviour*, 144(5), pp. 503–514. doi: 10.1163/156853907780713055.
- Pagel, M. D. (1999) 'Inferring the historical patterns of biological evolution.', *Nature*, 401(6756), pp. 877–884. doi: 10.1038/44766.
- Panhuis, T. M. and Wilkinson, G. S. (1999) 'Exaggerated male eye span influences contest outcome in stalk-eyed flies (Diopsidae)', *Behavioral Ecology and Sociobiology*, 46(4), pp. 221–227. doi: 10.1007/s002650050613.
- Parker, G. A. (1970) 'Sperm competition and its evolutionary consequences in the insects', *Biological Reviews*, 45(May), pp. 525–567. doi: 10.1111/j.1469-185X.1970.tb01176.x.
- Parker, G. A. (1984) *Sperm competition and the evolution of animal mating systems*. London: Academic Press.
- Parker, G. A., Baker, R. R. and Smith, V. G. F. (1972) 'The origin and evolution of gamete dimorphism and the male-female phenomenon', *Journal of Theoretical Biology*, 36(3), pp. 529–553. doi: 10.1016/0022-5193(72)90007-0.
- Partridge, L. and Farquhar, M. (1981) 'Sexual activity reduces lifespan of male fruitflies', *Nature*, pp. 580–582. doi: 10.1038/294580a0.
- Pearse, W. D., Cadotte, M. W., Cavender-Bares, J., Ives, A. R., Tucker, C. M., Walker, S. C. and Helmus, M. R. (2015) 'Pez: Phylogenetics for the environmental sciences', *Bioinformatics*, 31(17), pp. 2888–2890. doi: 10.1093/bioinformatics/btv277.
- Pellitteri-Rosa, D., Sacchi, R., Galeotti, P., Marchesi, M. and Fasola, M. (2011) 'Courtship Displays Are Condition-Dependent Signals That Reliably Reflect Male Quality in Greek Tortoises, Testudo graeca', *Chelonian Conservation and Biology*, 10(1), pp. 10–17. doi: 10.2744/CCB-0840.1.
- Peretti, A. V. and Eberhard, W. G. (2010) 'Cryptic female choice via sperm dumping favours male copulatory courtship in a spider', *Journal of Evolutionary Biology*, 23(2), pp. 271–281. doi: 10.1111/j.1420-9101.2009.01900.x.
- Petersson, E. (1987) 'Weight-associated male mating success in the swarming caddis fly, *Mystacides azureus* L.', *Annales Zoologici Fennici*, pp. 335–339.
- Phalan, B., Bertzky, M., Butchart, S. H. M., Donald, P. F., Scharlemann, J. P. W., Stattersfield, A. J. and Balmford, A. (2013) 'Crop Expansion and Conservation Priorities in Tropical Countries', *PLoS ONE*, 8(1). doi: 10.1371/journal.pone.0051759.
- Pilastro, A. and Bisazza, A. (1999) 'Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*)', *Proceedings of the Royal Society of London Series B-Biological Sciences*, 266(1431), pp. 1887–1891.

- Pilastro, A., Simonato, M., Bisazza, A. and Evans, J. P. (2004) 'Cryptic female preference for colorful males in guppies.', *Evolution; international journal of organic evolution*, 58(3), pp. 665–669. doi: 10.1111/j.0014-3820.2004.tb01690.x.
- Pitnick, S. and Brown, W. D. (2000) 'Criteria for demonstrating female sperm choice', *Evolution; international journal of organic evolution*, 54(3), pp. 1052–1056. doi: 10.1111/j.0014-3820.2000.tb00107.x.
- Pitnick, S., Brown, W. D. and Miller, G. T. (2001) 'Evolution of female remating behaviour following experimental removal of sexual selection', *Proceedings of the Royal Society of London Series B-Biological Sciences*, 268(1467), pp. 557–563. doi: 10.1098/rspb.2000.1400.
- Pitnick, S., Hosken, D. J. and Birkhead, T. R. (2009) 'Sperm morphological diversity', in Birkhead, T. R., Hosken, D. J., and Pitnick, S. (eds) *Sperm Biology: An Evolutionary Perspective*. Burlington: Elsevier, pp. 69–149.
- Pitnick, S., Miller, G. T., Reagan, J. and Holland, B. (2001) 'Males' evolutionary responses to experimental removal of sexual selection.', *Proceedings. Biological sciences / The Royal Society*, 268(1471), pp. 1071–80. doi: 10.1098/rspb.2001.1621.
- Pizzari, T. and Birkhead, T. R. (2000) 'Female feral fowl eject sperm of subdominant males.', *Nature*, 405, pp. 787–789. doi: 10.1038/35015558.
- Plesnar-Bielak, A., Skrzynecka, A. M., Prokop, Z. M. and Radwan, J. (2012) 'Mating system affects population performance and extinction risk under environmental challenge', *Proceedings of the Royal Society B: Biological Sciences*, 279(1747), pp. 4661–4667. doi: 10.1098/rspb.2012.1867.
- Pomfret, J. C. and Knell, R. J. (2006) 'Sexual selection and horn allometry in the dung beetle *Euoniticellus intermedius*', *Animal Behaviour*, 71(3), pp. 567–576. doi: 10.1016/j.anbehav.2005.05.023.
- Pomfret, J. C. and Knell, R. J. (2008) 'Crowding, sex ratio and horn evolution in a South African beetle community.', *Proceedings. Biological sciences / The Royal Society*, 275(1632), pp. 315–321. doi: 10.1098/rspb.2007.1498.
- Power, D. J. and Holman, L. (2015) 'Assessing the alignment of sexual and natural selection using radiomutagenized seed beetles', *Journal of Evolutionary Biology*, 28(5), pp. 1039–1048. doi: 10.1111/jeb.12625.
- Preston, B. T., Stevenson, I. R., Pemberton, J. M. and Wilson, K. (2001) 'Dominant rams lose out by sperm depletion.', *Nature*, 409(6821), pp. 681–682. doi: 10.1038/35055617.
- Promislow, D. (1992) 'Costs of sexual selection in natural populations of mammals', *Proceedings of the Royal Society B: Biological Sciences*, 247(1320), pp. 203–210.
- Promislow, D., Montgomerie, R. and Martin, T. E. (1992) 'Mortality costs of sexual dimorphism in birds', *Proceedings of the Royal Society B: Biological Sciences*, 250(1328), pp. 143–150. doi: 10.1098/rspb.1992.0142.
- Proulx, S. R. (1999) 'Matings Systems and the Evolution of Niche Breadth', *The American Naturalist*, 154(1), pp. 89–98. doi: 10.1086/303218.
- Proulx, S. R. (2002) 'Niche shifts and expansion due to sexual selection', *Evolutionary*

Ecology Research, 4(3), pp. 351–369.

R Development Core Team (2016) ‘R: A language and environment for statistical computing.’ Vienna, Austria: R Foundation for Statistical Computing.

Radwan, J. (2004) ‘Effectiveness of sexual selection in removing mutations induced with ionizing radiation’, *Ecology Letters*, 7(12), pp. 1149–1154. doi: 10.1111/j.1461-0248.2004.00681.x.

Radwan, J., Unrug, J., Śnigórska, K. and Gawrońska, K. (2004) ‘Effectiveness of sexual selection in preventing fitness deterioration in bulb mite populations under relaxed natural selection’, *Journal of Evolutionary Biology*, 17(1), pp. 94–99. doi: 10.1046/j.1420-9101.2003.00646.x.

Rahman, M. M., Turchini, G. M., Gasparini, C., Norambuena, F. and Evans, J. P. (2014) ‘The expression of pre- and postcopulatory sexually selected traits reflects levels of dietary stress in guppies’, *PLoS ONE*, 9(8), pp. 1–12. doi: 10.1371/journal.pone.0105856.

Rasmussen, J. L. (1994) ‘The influence of horn and body size on the reproductive behavior of the horned rainbow scarab beetle *Phanaeus difformis* (Coleoptera: scarabaeidae)’, *Journal of Insect Behavior*, 7(1), pp. 67–82. doi: 10.1007/BF01989828.

Reaney, L. T. and Knell, R. J. (2015) ‘Building a beetle: How larval environment leads to adult performance in a horned beetle’, *PLoS ONE*, 10(8), pp. 1–14. doi: 10.1371/journal.pone.0134399.

Reding, L., Swaddle, J. and Murphy, H. (2013) ‘Sexual selection hinders adaptation in experimental populations of yeast’, *Biology letters*, 9(March), pp. 9–13. doi: 10.1098/rsbl.2012.1202.

Reid, J. (1976) ‘Techniques’, in Heath, J. (ed.) *The Moths and Butterflies of Great Britain and Ireland. Vol. 1*. London: Curwen Press, pp. 117–132.

Revell, L. J. (2012) ‘phytools: An R package for phylogenetic comparative biology (and other things)’, *Methods in Ecology and Evolution*, 3(2), pp. 217–223. doi: 10.1111/j.2041-210X.2011.00169.x.

Rhein, M., Rintoul, S. R., Aoki, S., Campos, E., Chambers, D., Feely, R. A., Gulev, S., Johnson, G. C., Josey, S. A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L. D. and Wang, F. (2013) ‘Observations: Ocean’, in Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M. (eds) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, pp. 255–315.

Rice, W. R. (1996) ‘Sexually antagonistic male adaptation triggered by experimental arrest of female evolution.’, *Nature*, pp. 232–234. doi: 10.1038/381232a0.

Rice, W. R. (2000) ‘Dangerous liaisons’, *Proceedings of the National Academy of Sciences of the United States of America*, 97(24), pp. 12953–12955.

Roberts, K. E., Evison, S. E. F., Baer, B. and Hughes, W. O. H. (2015) ‘The cost of promiscuity: sexual transmission of *Nosema* microsporidian parasites in polyandrous honey bees’, *Scientific Reports*. Nature Publishing Group, 5(1), p. 10982. doi: 10.1038/srep10982.

- Rodd, F. H., Hughes, K. A., Grether, G. F. and Baril, C. T. (2002) 'A possible non-sexual origin of mate preference: are male guppies mimicking fruit?', *Proceedings of the Royal Society B: Biological Sciences*, 269(1490), pp. 475–481. doi: 10.1098/rspb.2001.1891.
- Rodríguez-Muñoz, R., Bretman, A. and Tregenza, T. (2011) 'Guarding males protect females from predation in a wild insect', *Current Biology*, 21(20), pp. 1716–1719. doi: 10.1016/j.cub.2011.08.053.
- Rowe, L., Arnqvist, G., Sih, A. and Krupa, J. J. (1994) 'Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system', *Trends in Ecology & Evolution*, 9, pp. 289–293.
- Rowe, L. and Houle, D. (1996) 'The Lek Paradox and the Capture of Genetic Variance by Condition Dependent Traits', *Proceedings of the Royal Society B: Biological Sciences*, 263(1375), pp. 1415–1421. doi: 10.1098/rspb.1996.0207.
- Rundle, H. D., Chenoweth, S. F. and Blows, M. W. (2006) 'The Roles of Natural and Sexual Selection during Adaptation to a Novel Environment', *Evolution*, 60(6011), pp. 2218–2225. doi: 10.1111/j.0014-3820.2006.tb01859.x.
- Sakaluk, S. K. (1991) 'Post-copulatory mate guarding in decorated crickets', *Animal Behaviour*, 41(2), pp. 207–216. doi: 10.1016/S0003-3472(05)80472-5.
- Schärer, L., Janicke, T. and Ramm, S. A. (2014) 'Sexual Conflict in Hermaphrodites Sexual Conflict in Hermaphrodites', *Cold Spring Harbor Perspective Biology*, pp. 1–26. doi: 10.1101/cshperspect.a017673.
- Schneider, J. M. (2014) 'Sexual Cannibalism as a Manifestation of Sexual Conflict', *Cold Spring Harbor Perspectives in Biology*, 6(11). doi: 10.1101/cshperspect.a017731.
- Schwagmeyer, P. L. and Parker, G. A. (1990) 'Male mate choice as predicted by sperm competition in 13-lined ground squirrels', *Nature*, 348, pp. 62–64.
- Scordato, E. S. C., Bontrager, A. L. and Price, T. D. (2012) 'Cross-generational effects of climate change on expression of a sexually selected trait', *Current Biology*. Elsevier Ltd, 22(1), pp. 78–82. doi: 10.1016/j.cub.2011.11.044.
- Seeley, C. and Dukas, R. (2011) 'Teneral matings in fruit flies: Male coercion and female response', *Animal Behaviour*. Elsevier Ltd, 81(3), pp. 595–601. doi: 10.1016/j.anbehav.2010.12.003.
- Sheldon, B. C. (2000) 'Differential allocation: Tests, mechanisms and implications', *Trends in Ecology and Evolution*, pp. 397–402. doi: 10.1016/S0169-5347(00)01953-4.
- Shine, R. (1978) 'Sexual size dimorphism and male combat in snakes', *Oecologia*, 33(3), pp. 269–277. doi: 10.1007/BF00348113.
- Shine, R. (1994) 'Sexual Size Dimorphism in Snakes Revisited', *Copeia*, 1994(2), p. 326. doi: 10.2307/1446982.
- Siller, S. (2001) 'Sexual selection and the maintenance of sex.', *Nature*, 411(6838), pp. 689–692. doi: 10.1038/35079578.
- Da Silva, P. G. and Hernández, M. I. M. (2015) 'Spatial patterns of movement of dung beetle

- species in a tropical forest suggest a new trap spacing for dung beetle biodiversity studies', *PLoS ONE*, 10(5), pp. 1–18. doi: 10.1371/journal.pone.0126112.
- Simmons, L. W. (1987) 'Female choice in the field cricket *Gryllus bimaculatus* (De Geer)', *Behavioral Ecology and Sociobiology*, 21, pp. 313–321. doi: 10.1016/S0003-3472(86)80217-2.
- Simmons, L. W. (2001) *Sperm Competition and its Evolutionary Consequences in the insects*. New Jersey: Princeton University Press.
- Simmons, L. W. (2003) 'The evolution of polyandry : patterns of genotypic variation in female mating frequency , male fertilization success and a test of the sexy-sperm hypothesis', *Journal of evolutionary biology*, 16, pp. 624–634. doi: 10.1046/j.1420-9101.2003.00572.x.
- Simmons, L. W. (2005) 'The Evolution of Polyandry: Sperm Competition, Sperm Selection, and Offspring Viability', *Annual Review of Ecology, Evolution, and Systematics*, 36(1), pp. 125–146. doi: 10.1146/annurev.ecolsys.36.102403.112501.
- Simmons, L. W. and Bailey, W. J. (1990) 'Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera: Tettigoniidae)', 44(7), pp. 1853–1868.
- Simmons, L. W., Emlen, D. J. and Tomkins, J. L. (2007) 'Sperm competition games between sneaks and guards: A comparative analysis using dimorphic male beetles', *Evolution*, 61(11), pp. 2684–2692. doi: 10.1111/j.1558-5646.2007.00243.x.
- Simmons, L. W. and Garcia-Gonzalez, F. (2008) 'Evolutionary Reduction in Testes Size and Competitive Fertilization Success in Response To the Experimental Removal of Sexual Selection in Dung Beetles', *Evolution*, 62(10), pp. 2580–2591. doi: 10.1111/j.1558-5646.2008.00479.x.
- Simmons, L. W. and García-González, F. (2008) 'Evolutionary reduction in testes size and competitive fertilization success in response to the experimental removal of sexual selection in dung beetles', *Evolution*, 62(10), pp. 2580–2591. doi: 10.1111/j.1558-5646.2008.00479.x.
- Simmons, L. W. and Kotiaho, J. S. (2002) 'Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits.', *Evolution; international journal of organic evolution*, 56(8), pp. 1622–1631. doi: 10.1554/0014-3820(2002)056[1622:EOEPOP]2.0.CO;2.
- Simmons, L. W., Tomkins, J. L. and Hunt, J. (1999) 'Sperm competition games played by dimorphic male beetles', *Proceedings of the Royal Society B: Biological Sciences*, 266(October 1998), pp. 145–150. doi: 10.1098/rspb.1999.0614.
- Simons, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. (1994) 'Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers', *Annals of the Entomological Society of America*, pp. 651–701. doi: 10.1080/17470210902990829.
- Slade, E. M., Mann, D. J. and Lewis, O. T. (2011) 'Biodiversity and ecosystem function of tropical forest dung beetles under contrasting logging regimes', *Biological Conservation*. Elsevier Ltd, 144(1), pp. 166–174. doi: 10.1016/j.biocon.2010.08.011.
- Slade, E. M., Mann, D. J., Villanueva, J. F. and Lewis, O. T. (2007) 'Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem

- function in a tropical forest', *Journal of Animal Ecology*, 76(6), pp. 1094–1104. doi: 10.1111/j.1365-2656.2007.01296.x.
- Snook, R. R. and Karr, T. L. (1998) 'Only long sperm are fertilization-competent in six sperm-heteromorphic *Drosophila* species.', *Current biology : CB*, 8(5), pp. 291–294. doi: 10.1016/S0960-9822(98)70112-5.
- Sodhi, N. S., Koh, L. P., Brook, B. W. and Ng, P. K. L. (2004) 'Southeast Asian biodiversity: An impending disaster', *Trends in Ecology and Evolution*, 19(12), pp. 654–660. doi: 10.1016/j.tree.2004.09.006.
- Sorci, G., Møller, A. P. and Clobert, J. (1998) 'Plumage dichromatism of birds predicts introduction success in New Zealand', *Journal of Animal Ecology*, 67(2), pp. 263–269. doi: 10.1046/j.1365-2656.1998.00199.x.
- Sowig, P. (1996) 'Duration and benefits of biparental brood care in the dung beetle *Onthophagus vacca* (Coleoptera: Scarabaeidae)', *Ecological Entomology*, 21(September), pp. 81–86. doi: 10.1017/CBO9781107415324.004.
- Spangler, Hayward, G., Greenfield, M. D. and Takessian, A. (1984) 'Ultrasonic mate calling in the lesser wax moth', *Physiological Entomology*, 9(1), pp. 87–95. doi: 10.1111/j.1365-3032.1984.tb00684.x.
- Stamatakis, A. (2006) 'RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models', *Bioinformatics*, 22(21), pp. 2688–2690. doi: 10.1093/bioinformatics/btl446.
- Stöver, B. C. and Müller, K. F. (2010) 'TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses.', *BMC bioinformatics*, 11(7), p. 9. doi: 10.1186/1471-2105-11-7.
- Stutt, A. D. and Siva-Jothy, M. T. (2001) 'Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*.', *Proceedings of the National Academy of Sciences of the United States of America*, 98(10), pp. 5683–5687. doi: 10.1073/pnas.101440698.
- Sutter, A., Simmons, L. W., Lindholm, A. K. and Firman, R. C. (2016) 'Function of copulatory plugs in house mice: Mating behavior and paternity outcomes of rival males', *Behavioral Ecology*, 27(1), pp. 185–195. doi: 10.1093/beheco/arv138.
- Tanaka, Y. (1996) 'Sexual selection enhances population extinction in a changing environment.', *Journal of theoretical biology*, 180(3), pp. 197–206. doi: 10.1006/jtbi.1996.0096.
- Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) 'CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice', *Nucleic Acids Research*, 22(22), pp. 4673–4680. doi: 10.1093/nar/22.22.4673.
- Tomkins, J. L., Hazel, W. N., Penrose, M. A., Radwan, J. and Lebas, N. R. (2011) 'Habitat complexity drives experimental evolution of a conditionally expressed secondary sexual trait', *Current Biology*. Elsevier Ltd, 21(7), pp. 569–573. doi: 10.1016/j.cub.2011.02.032.
- Tomkins, J. L., Radwan, J., Kotiaho, J. S. and Tregenza, T. (2004) 'Genic capture and resolving the lek paradox', *Trends in Ecology and Evolution*, pp. 323–328. doi:

10.1016/j.tree.2004.03.029.

Tomkins, J. L. and Simmons, L. W. (2000) 'Sperm competition games played by dimorphic male beetles: fertilization gains with equal mating access.', *Proceedings. Biological sciences / The Royal Society*, 267(1452), pp. 1547–53. doi: 10.1098/rspb.2000.1177.

Tregenza, T. and Wedell, N. (2000) 'Genetic compatibility, mate choice and patterns of parentage: Invited review', *Molecular Ecology*, pp. 1013–1027. doi: 10.1046/j.1365-294X.2000.00964.x.

Trematerra, P. and Pavan, G. (1995) 'Ultrasound production in the courtship behaviour of *Ephestia cautella* (Walk.), *E. kuehniella* Z. and *Plodia interpunctella* (Hb.) (Lepidoptera: Pyralidae)', *Journal of Stored Products Research*, 31(1), pp. 43–48. doi: 10.1016/0022-474X(94)00034-Q.

Trivers, R. L. (1972) 'Parental investment and sexual selection', in Cambell, B. (ed.) *Sexual selection and the descent of man, 1871-1971*. Chicago: Aldine, pp. 136–179.

Uhl, G., Nessler, S. H. and Schneider, J. M. (2009) 'Securing paternity in spiders? A review on occurrence and effects of mating plugs and male genital mutilation', *Genetica*, 138(1), pp. 75–104. doi: 10.1007/s10709-009-9388-5.

Vahed, K. (1998) 'The function of nuptial feeding in insects: review of empirical studies', *Biological Reviews of the Cambridge Philosophical Society*, 73(1), pp. 43–78. doi: 10.1017/s0006323197005112.

Vahed, K. and Parker, D. J. (2012) 'The Evolution of Large Testes: Sperm Competition or Male Mating Rate?', *Ethology*, 118(1), pp. 107–117. doi: 10.1111/j.1439-0310.2011.01991.x.

Vanpé, C., Morellet, N., Kjellander, P., Goulard, M., Liberg, O. and Hewison, A. J. M. (2009) 'Access to mates in a territorial ungulate is determined by the size of a male's territory, but not by its habitat quality', *Journal of Animal Ecology*, 78(1), pp. 42–51. doi: 10.1111/j.1365-2656.2008.01467.x.

Vega-Trejo, R., Jennions, M. D. and Head, M. L. (2016) 'Are sexually selected traits affected by a poor environment early in life?', *BMC Evolutionary Biology*. BMC Evolutionary Biology, 16(1), p. 263. doi: 10.1186/s12862-016-0838-2.

Vehrencamp, S. L., Ellis, J. M., Cropp, B. F. and Koltz, J. M. (2014) 'Negotiation of territorial boundaries in a songbird', *Behavioral Ecology*, 25(6), pp. 1436–1450. doi: 10.1093/beheco/aru135.

Vergara, P., Martinez-Padilla, J., Mougeot, F., Leckie, F. and Redpath, S. M. (2012) 'Environmental heterogeneity influences the reliability of secondary sexual traits as condition indicators', *Journal of Evolutionary Biology*, 25(1), pp. 20–28. doi: 10.1111/j.1420-9101.2011.02399.x.

Ward, P. I. (2001) 'Cryptic female choice in the yellow dung fly *Scatophaga stercoraria* (L.)', *Evolution*, 54(5), pp. 1680–1686. doi: 10.1111/j.0014-3820.2000.tb00712.x.

Watanabe, M., Bon'no, M. and Hachisuka, A. (2000) 'Eupyrene sperm migrates to spermatheca after apyrene sperm in the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae)', *Journal of Ethology*, 18(2), pp. 91–99. doi: 10.1007/s101640070006.

- Wearn, O. R., Carbone, C., Rowcliffe, J. M., Bernard, H. and Ewers, R. M. (2016) 'Grain-dependent responses of mammalian diversity to land use and the implications for conservation set-aside', *Ecological Applications*, 26(5), pp. 1409–1420. doi: 10.1890/15-1363.
- Wedell, N. and Cook, P. A. (1999) 'Butterflies tailor their ejaculate in response to sperm competition risk and intensity', *Proceedings of the Royal Society B: Biological Sciences*, 266(1423), p. 1033. doi: 10.1098/rspb.1999.0740.
- Wedell, N., Gage, M. J. G. and Parker, G. A. (2002) 'Sperm competition, male prudence and sperm-limited females', *Trends in Ecology and Evolution*, 17(7), pp. 313–320. doi: 10.1016/S0169-5347(02)02533-8.
- Wedell, N. and Ritchie, M. G. (2004) 'Male age, mating status and nuptial gift quality in a bushcricket', *Animal Behaviour*, 67(6), pp. 1059–1065. doi: 10.1016/j.anbehav.2003.10.007.
- West, P. M. and Packer, C. (2002) 'Sexual selection, temperature, and the lion's mane.', *Science (New York, N.Y.)*, 297(5585), pp. 1339–1343. doi: 10.1126/science.1073257.
- Whipple, S. D. and Hoback, W. W. (2012) 'A Comparison of Dung Beetle (Coleoptera: Scarabaeidae) Attraction to Native and Exotic Mammal Dung', *Environmental Entomology*, 41(2), pp. 238–244. doi: 10.1603/EN11285.
- Whitlock, M. C. (2000) 'Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection.', *Evolution; international journal of organic evolution*, 54(6), pp. 1855–1861. doi: 10.2144/05384CI01.
- Whitlock, M. C. and Agrawal, A. F. (2009) 'Purging the genome with sexual selection: Reducing mutation load through selection on males', *Evolution*, 63(3), pp. 569–582. doi: 10.1111/j.1558-5646.2008.00558.x.
- Wigby, S. and Chapman, T. (2004) 'Female resistance to male harm evolves in response to manipulation of sexual conflict.', *Evolution; international journal of organic evolution*, 58(5), pp. 1028–1037. doi: 10.1111/j.0014-3820.2004.tb00436.x.
- Wilkinson, G. S. and Reillo, P. R. (1994) 'Female Choice Response to Artificial Selection on an Exaggerated Male Trait in a Stalk-Eyed Fly', *Proceedings of the Royal Society B: Biological Sciences*, 255(1342), pp. 1–6. doi: 10.1098/rspb.1994.0001.
- Williams, S. E., Moritz, C., Shoo, L. P., Isaac, J. L., Hoffmann, A. A. and Langham, G. (2008) 'Towards an Integrated Framework for Assessing the Vulnerability of Species to Climate Change', *PLoS Biol*, 6(12), pp. 2621–2626. doi: 10.1371/journal.pbio.0060325.
- Willis, S. (2015) *Sexual Conflict and Sexual Selection in the Indian Meal Moth, Plodia interpunctella*. University of Exeter.
- Wong, B. B. M. and McCarthy, M. (2009) 'Prudent male mate choice under perceived sperm competition risk in the eastern mosquito fish', *Behavioral Ecology*, 20(2), pp. 278–282. doi: 10.1093/beheco/arp010.
- Xu, J. and Wang, Q. (2010) 'Mechanisms of last male precedence in a moth: Sperm displacement at ejaculation and storage sites', *Behavioral Ecology*, 21(4), pp. 714–721. doi: 10.1093/beheco/arq044.

Zahavi, A. (1975) 'Mate selection-A selection for a handicap', *Journal of Theoretical Biology*, 53(1), pp. 205–214. doi: 10.1016/0022-5193(75)90111-3.

Zhu, J., Ryne, C., Unelius, C. R., Valeur, P. G. and Löfstedt, C. (1999) 'Reidentification of the female sex pheromone of the Indian meal moth, *Plodia interpunctella*: Evidence for a four-component pheromone blend', *Entomologia Experimentalis et Applicata*, 92(2), pp. 137–146. doi: 10.1023/A:1003703724011.

Zuk, M., Rotenberry, J. T. and Tinghitella, R. M. (2006) 'Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets', *Biology Letters*, 2(4), pp. 521–524. doi: 10.1098/rsbl.2006.0539.